Humanized yeast genetic interaction mapping predicts synthetic lethal interactions of FBXW7 in breast cancer

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Abstract

A Synthetic lethal (SL) interaction involves a pair of genes (geneA and geneB) where inhibition of either geneA or geneB individually has no effect on cell viability, but the inhibition of both geneA and geneB causes cell death. SL interactions that occur between gene pairs can be exploited for cancer therapeutics. Studies in the model eukaryote yeast have identified approximately 550,000 negative genetic interactions that have been extensively applied to characterize novel pathways and gene functions. In the context of this thesis, a negative genetic interaction is the equivalent of a SL interaction. Harnessing the vast available knowledge of yeast genetics, we generated a Humanized Yeast Genetic Interaction Network (HYGIN) for 1,009 human genes with yeast orthologs and 10,419 interactions. Through the addition of patient-data from The Cancer Genome Atlas (TCGA), we generated a breast cancer specific subnetwork. Specifically, by comparing 1,009 genes in HYGIN to genes that were down-regulated in breast cancer, we identified 15 breast cancer genes with 130 potential SL interactions. Interestingly, 32 of the 130 predicted SL interactions occurred with *FBXW7*, a well-known tumor suppressor that functions as a substrate-recognition protein within the SKP/CUL1/F-Box ubiquitin ligase complex for degradation through the proteasome. Validation of these SL interactions using chemical genetic data indicate that patients with loss of *FBXW7* may respond to treatment with drugs like Selumitinib or Cabozantinib. Taken together, our patient-data driven interpretation of HYGIN represents a novel strategy to uncover therapeutically relevant drug targets.

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to my parents

Contents

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List of Abbreviations

APC/c	anaphase-promoting cyclosome complex
APC	adenomatous polyposis coli
ARF	alternate reading frame
ATM	ataxia-telangiectasia-mutated gene product
BL1	basal-like 1
BL2	basal-like 2
BLAST	basic local alignment search tool
BLIA	basal-like immune-activated
BLIS	basal-like immune-uppressed
BRCA	breast cancer protein
CD95	cluster of differentiation 95
CDC	cell division cycle
CCLE	cancer cell line encyclopedia
CPD	CDC4 phospho-degron motifs
CRISPR	clustered regularly interspaced short palindromic repeats
DAISY	data mining synthetic lethality
DECIPHER	database of genomic variation and phenotype in humans using ensembl resources
DNA	deoxyribonucleic acid
ER	estrogen rceptor
Fast-SL	fast synthetic lethal
FBA	flux balance analysis
FBXW7	F-box and WD repeat domain-containing 7
GO	gene ontology
HER2	human epidermal growth factor rceptor 2
HYGIN	humanized yeast genetic interaction network
IM	immunomodulatory
LAR	luminal androgen receptor
M	mesenchymal
MiSL	mining synthetic lethals
MSL	stem-like
PESCADOR	platform for exploration of significant concepts associated to co-occurrence relationships
PPP	pentose phosphate pathway
PR	progesterone rceptor
RNA	ribonucleic acid
RNA-seq	RNA-sequencing
RPE	ribulose-5-phosphate-3-epimerase
RSEM	RNA-seq by expectation maximization
SCNA	single copy number alterations

SCF	SKP/CUL1/F-Box) E3 ubiquitin ligase complex
SDL	synthetic dosage lethality
shRNA	short hairpin RNA
siRNA	small interfering RNA
SKP1	S-phase kinase associated protein 1
SL	synthetic lethality
SoF	survival of the fittest
SynLethDB	synthetic lethal database
T-ALL	T-cell acute lymphoblastic leukaemia
TCGA	the cancer genome atlas
TNBC	triple-negative breast cancer
TSG	tumour suppressing genes
UND	unstable
UniProt	universal protein reference

Chapter 1

Introduction

Genetically, cancer is a complex disease with no two patients exhibiting the same genetic profiles of tumors. Recent advances in tumor sequencing and identification of key driver genes has allowed the development of more targeted treatment strategies by leveraging individual patient genetics [10]. However, the druggability of these targets becomes challenging if these genes are not expressed or are down-regulated in certain cancers.

Synthetic lethality takes advantage of functional genetic interactions between gene pairs to develop targeted therapies. Genetic interactions are the phenomenon where two or more gene products interact with one another that results in a deviation of the expected phenotype [78]. Synthetic lethal (SL) interactions are beginning to be appreciated as a method for identifying druggable targets [30, 89, 101]. Inhibiting the synthetic lethal partner with a therapeutic drug selectively eliminates cancer cells leaving normal cells largely unaffected. By exploiting these synthetic lethal interactions, we can maximize the efficiency of personalized treatment options (novel drugs, or repurposed drugs) and, potentially, minimize the side effects that a patient experiences as a result of the therapy. Although the advent of genome-wide shRNA and CRISPR-Cas9 screens have provided the specificity needed to perform the comprehensive epistasis mapping on any number of chosen gene pairs [48, 90, 127], experimentally testing all possible gene pairs across multiple cell types to identify SL is laborious and time-consuming.

Previously, yeast SL genetic interaction data has been used to identify SL gene interactions in humans [27, 76, 113]. These yeast-directed approaches have helped uncover several druggable interactions in human cells. For example, McManus *et al.* identified the first humanized synthetic lethal interactions of *RAD54B*-deficient human colorectal cancer cells by mapping the corresponding yeast synthetic lethal interactions to human cancer cells [76]. Thus, rather than evaluating all human SL interactions, evaluating some in a yeast context

provided valuable insights into SL interactions in cancer. This approach reduces the dimensionality of searching for SL interactions in humans by reducing the total set of potential genes that can interact with one another to only those that have yeast orthologs. As a result, since the yeast genome is substantially smaller than the human genome and only a fraction of yeast genes have human orthologs, the total sample space for potential SL interactions in humans is reduced.

Although using pre-existing experimental data from yeast is extremely useful, results generated from mapping yeast data to humans does not provide a comprehensive picture of the human SL landscape. It turns out that the greatest strength of using yeast data to identify human SL interactions (reducing the dimensionality of the problem) is also it's greatest weakness: eliminating the majority of human genes from the analysis. The human genome contains approximately 20,000 genes and the yeast genome approximately 6,000. Given the evolutionary distance between yeast and humans, it is not surprising that there are only around 2,000 yeast genes that have human orthologs. As a result, if we are at most examining these 2,000 human genes with yeast orthologs for SL interactions, we are only evaluating 1/10 of the human genome. Therefore, using model species such as yeast is extremely useful for advancing the discovery of novel SL interactions in humans; however, caution must be taken when interpreting the results as there could exist many SL interactions between human genes without yeast orthologs.

The Cell Map contains a global genetic interaction map for *Saccharomyces cerevisiae* [23]. Using more than 23 million experimentally generated double mutants, approximately 550,000 negative and 350,000 positive interactions were reported in The Cell Map. Previous work by Deshpande *et al.* made use of an older version of this data [22], and was motivation for this study. Having identified potential problems with the Deshpande *et al.* work, this thesis aimed to improve upon their strategy for generating a human network of potential SL interactions based on previously experimentally validated yeast interaction data. Harnessing the vast available knowledge of yeast genetics and using yeast-human ortholog mapping, we generated a humanized yeast genetic interaction network (HYGIN) that has the potential to identify novel cancer-specific synthetic lethal interactions and novel cancer treatment strategies. Since no cancer data has been used in the generation of HYGIN any

cancer specific data can be incorporated to generate a subset of SL interactions in humans that are cancer specific. Breast cancer is the third most common type of cancer in Canada accounting for more than 25% of all new cases of cancer in women and 13% of all cancers in Canada. This network is then integrated with patient data from The Cancer Genome Atlas (TCGA) (`https://cancergenome.nih.gov/`) to identify genes that are down-regulated in breast cancer to provide a breast cancer-focused version of HYGIN. The breast cancer specific subnetwork contains 130 SL interactions with 15 genes that were identified to be down-regulated in breast cancer and 115 other genes in HYGIN. Of these 130 SL interactions, we predict 32 novel synthetic lethal interactions of the tumor suppressor *FBXW7* that could be exploited for patient-specific cancer therapeutics using previously identified or novel drugs.

The remainder of this thesis is organized as follows. Background information on cancer, yeast as a model organism, synthetic lethality, computational approaches to synthetic lethality, data sources for computational studies and *FBXW7* is given in Chapter 2. Chapter 3 articulates the research hypothesis of this thesis. The data used and methodology for generating HYGIN, initial data analysis techniques, statistical analyses used, validation of the network, and drug analyses are covered in Chapter 4. Results from evaluating HYGIN and the breast cancer subnetwork are presented in Chapter 5. Chapter 6 analyzes and interprets the results present in Chapter 5, discusses the shortcomings of previous work and compares HYGIN to it. Finally, Chapter 7 summarizes the conclusions of this work and proposes future research endeavours using HYGIN. Currently a manuscript describing this work is in the final stages of preparation for journal publication. The majority of the manuscript is integrated into this thesis document as Chapter 1, Sections 4.1 – 4.3, Sections 4.5 – 4.6, Chapter 5, portions of Section 6.1 and Section 6.4.

Chapter 2

Background

This section provides the necessary background material for understanding the research that is present in this thesis. Section 2.1 provides a brief introduction to cancer. Section 2.1.1 gives some background on the molecular classification of cancers, and section 2.1.2 provides an overview of information pertaining to breast cancer. Section 2.2 gives an introduction to yeast as a model organism, and section 2.2.1 discusses orthology and different ways that genes can be mapped between species. Section 2.3 contains a short background on synthetic lethality, it's applications and previous work. A survey of previous computational approaches to synthetic lethality can be found in section 2.4. Section 2.5 provides background information on the sources of data used in this thesis: 2.5.1; the Cell Map, 2.5.2; InParanoid, 2.5.3; SynLethDB, 2.5.4; the cancer genome atlas. Finally, section 2.6 provides an introduction to the tumor suppressor FBXW7 and it's role in cancer.

2.1 Cancer

Cancer is an extremely complex group of diseases that are the result of loss of control over cell division and cell growth. Recent statistics from the Canadian Cancer Society suggest that 1 in 2 Canadians will develop cancer in their lifetime, and 1 in 4 will die from it [107]. With such a high incidence rate in the population, it is necessary to identify and understand the mechanisms of cancer so that patients can receive better treatment. Since there are many different ways that a healthy cell can transform into a tumor cell, the identification of drug targets and personalized therapeutics is difficult. As a result, researchers are leveraging genetics research to develop personalized approaches to cancer therapy.

There are many different ways that cancer can arise in a cell leading to the complicated

task of identifying which genes are associated with which cancers. With advances in molecular biology techniques and genetics, researchers have identified two major types of genes that contribute to cancer: tumor suppressor genes (TSGs) and oncogenes. TSGs are a class of genes that under normal cellular function can repair damaged DNA (e.g. ataxia-telangiectasia-mutated gene product (*ATM*), breast cancer protein (*BRCA*), and *p53*), regulate cell division (e.g. alternate reading frame (*ARF*), *RIZ1*, *p27*, and *p53*), and control apoptosis (regulated cell death) (e.g. adenomatous polyposis coli (APC), cluster of differentiation 95 (*CD95*), and *p53*) [115]. However, in cases where TSGs are mutated or deleted these cell processes are no longer regulated and can lead to cancer. A classic example of a TSG is the nuclear transcription factor *p53* [87]. Genetic analysis of multiple cancer types suggests that *p53* has a loss of function mutation in more than 50% of cancers [5, 54, 82, 116]. In these cases, p53 loses the ability to repair damaged DNA and initiate cell apoptosis. There have even been studies in mice that show that mice with mutations to *p53* spontaneously develop tumors [31]. There are several different genetic means by which genes can lose expression and lead to cancer: mutations, deletions in the upstream region responsible for the regulation of a gene, whole gene deletions due to deletions on a chromosomal level, siRNA's, and most prominently DNA methylation resulting in a loss of gene expression [9, 33, 60, 79, 132].

On the other hand, oncogenes are the key drivers of tumor growth and are often expressed at high levels in tumor cells [67]. Oncogenes can cause chromosome instability and unscheduled cell growth and have been shown not only to be involved in cancer development, but also in cancer maintenance [125]. They have also been shown to play an important role in cancer cell metabolism where they aid tumors in meeting their nutritional retirements for growth, contributing to the notion that cancer is a metabolic disease [80]. However, oncogenes have to be activated in order to cause cancer. Proto-oncogenes are normally expressed genes in the cell that have the capacity to cause cancer when they are activated to become oncogenes [121]. For example, under normal cellular conditions, the highly regulated *MYC* (a transcription factor) proto-oncogene does not have the capacity to initiate cancer development. However, a mutation within the regulatory region for *MYC* could increase its expression making it an oncogene [25].

2.1.1 Molecular classification of cancers

Cancers tend to be classified based on the tissue or cell type that they were isolated from (e.g. breast, prostate, lung, esophageal, or pancreatic cancers). Though this method has been useful for doctors, patients, and researchers in the past, recent advances in genome sequencing technology have shed light onto the genetics of these cancers and potentially provided a complimentary means of classifying them for treatment [24, 53]. A recent study has shown that 43% of cancer cases on average are more similar to cancers from other tissue types than they are similar to other cancers from the same tissue [24]. For example, Heim *et. al* report that 14% of breast cancer cases are more closely related to ovarian cancer than they are to breast cancer. Furthermore, another study by Hoadley *et al* examined 12 cancer types and identified 11 major classification subtypes based on genetic analysis [53]. Although 5 of the subtypes contained cancer cases that were almost exactly the same as classifying cancers by tissue, it was found that cases of bladder cancer could be split into three different subtypes [53]. Classifying cancers into types based on genetics rather than tissue type provides a novel categorization for researchers that hopefully results in the re-purposing of cancer therapeutics from tissue types of cancer to molecular types.

Hoadley *et al.* released a more recent article in 2018 that expanded on their previous research and included 33 types of cancer and more than 10,000 tumors [52]. The updated research concluded that molecular tumor classification is influenced by cell-of-origin, but is not determined by it [52]. It is important to maintain the current cell-of-origin classification system for cancer as it allows researchers and doctors to understand what is happening in the body and how it is affecting physiology in a patient. However, molecular classification systems are also important as they provide doctors with novel treatment strategies that might not have been identified through cell-of-origin typing. Ultimately, neither of these two classifications systems is perfect; both have their merits and are necessary for prioritizing patient care and effective treatment.

Although cancer therapies have come a long way since they were first introduced, there are still negative side effects that impact patients undergoing treatment. As a result, there is a need for new therapeutics that specifically target cancer cells, while minimizing negative

side effects. By grouping cancers based on their genetic similarities, researchers can use existing therapeutic strategies that target a well know cancer-associated gene in one cancer and apply it to genetically similar cancers. Research efforts towards patient-specific therapies often evaluate known therapeutics and their effectiveness for patients with specific genetic profiles or biomarkers. This is an enticing research avenue that can potentially reduce the negative side effects of treatment and eliminates superfluous, ineffective treatments.

With advances in genome sequencing technology and the ability to identify cancer biomarkers through genome sequencing, personalized medicine is now becoming a reality. Patient-specific therapeutics are starting to become common practice in many cancer treatment strategies [124, 131]. These approaches involve selecting therapeutics based on the genetic profiles of the patients and their tumor(s). Identifying patient-specific novel druggable targets in cancer is still costly with respect to time and resources. As a result, research efforts are turning to computational techniques to predict patient specific druggable genes for efficient cancer therapeutics.

2.1.2 Breast cancer

Breast cancer is the third most common form of cancer in Canada, accounting for more than 25% of new cases of cancer in women in 2017 [107]. There are 5 intrinsic types of breast cancer: luminal A, luminal B, triple-negative breast cancer (TNBC), human epidermal growth factor receptor 2 enriched (HER2-enriched), and normal-like [14, 85, 93, 110]. Luminal A breast cancer is characterized by the presence of the estrogen receptor (ER) and the progesterone receptor (PR), the absence of HER2 (abbreviated ER+/PR+/HER2-), and low levels of the Ki-67 protein. This type of breast cancer has the best prognosis among breast cancers [85]. Luminal B breast cancer is ER+/PR+, either HER2 positive or negative (ER+/PR+/HER2+/-) and has high levels of Ki-67 [85]. In contrast, TNCB is ER-/PR-/HER2- and represents 10-20% of breast cancers [135]. HER2-enriched breast cancer is ER-/PR-/HER2+ and tends to grow faster and have a worse prognosis than luminal A and luminal B breast cancers [85]. Finally, normal-like breast cancer is ER+/PR+/HER2- and very similar to luminal A except it has a slightly worse prognosis than luminal A breast cancer [85]. Within each type of breast cancer mentioned above, there are various sub-types defined by their molecular profiles. Of

particular interest due to its high prevalence among women in Canada is TNBC.

Over the past decade, researchers have been working to identify the molecular subtypes of TNBC. In 2011, Lehmann *et al.* published a study that identified six molecular subtypes of TNCB: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) [68]. However, in 2016, Lehmann updated their classification system because transcripts previously described in IM samples were from infiltrating lymphocytes, and transcripts from MSL samples were actually from tumor-associated stromal cells [69]. As as a result, the new classification system that they propose contains only four of the above mentioned molecular subtypes of TNCB: BL1, BL2, M, and LAR [69].

Interestingly, between the publishing of the Lehmann 2011 [68] and Lehmann 2016 [69] manuscripts, three other research groups published different molecular classification systems for TNBC [16, 59, 73]. Masuda *et al.* reported seven subtypes in 2013: BL1, BL2, M, IM, MSL, LAR, and unstable (UNS) [73]. Burstein *et al.* 2015 reported four molecular subtypes which they named LAR, mesenchymal (MES), basal-like immune-uppressed (BLIS), and basal-like immune-activated (BLIA) [16]. In contrast, Jezequel *et al.* identified three molecular subtypes: LAR, and two different types of basal-like (one with high M2-like macrophages and a low immune response, and the other with low M2-like macrophages and a high immune response [59]).

There is an unsurmountable amount of evidence that strongly suggests that TNBC is heterogeneous and made up of several different subtypes. Although there does not seem to be a consensus on the molecular subtypes within the research community, it is reassuring that most studies are identifying some of the same subtypes: basal-like, MES, and LAR. Identifying molecular subtypes of breast cancer has broader applications for the cancer research community. By expanding molecular subtyping analyses to multiple cancer types, researchers can group cancers based on genetics rather than cell-of-origin. The hope is that this novel method of classification will group cancers into types that are treatable by repurposing existing therapeutics and increase patient care.

2.1.3 The tumor suppressor FBXW7

FBXW7 (F-box and WD repeat domain-containing 7) is the substrate recognition component of the SCF (SKP/CUL1/F-Box) E3 ubiquitin ligase complex that has several aliases: *FBW7*, *AGO*, *CDC4*, and *SEL10* (Figure 2.1) [130]. FBXW7 has been classified as a tumor suppressor [130], and studies have shown that loss of *FBXW7* gene expression results in chromosomal instability through deregulation of its protein substrates, including cyclin E [111]. When conserved CDC4 phospho-degron motifs (CPDs) on target proteins are phosphorylated, FBXW7 binds to them and the protein substrate is targeted for ubituitination through the SCF complex. Because the substrates of FBXW7 are involved with cell division, cell growth, and cell differentiation, phosphorylation of these substrates by glycogen synthase kinase 3 (GSK3) is highly regulated [26]. The proteins that FBXW7 interacts with include several well known oncogenes including cyclin E, JUN, Notch, and MYC [130].

The *FBXW7* gene is located on human chromosome 4q32, a location that is often deleted in cancers [112]. Because FBXW7 is part of the SCF E3 ubiquitin ligase complex, in cancers where it is deleted, its oncogenic substrates become excess in the cell. For this reason, FBXW7 was proposed to function as a tumor suppressor [129]. It has also been shown that *FBXW7* is mutated in around 6% of cancer tumors but with a large amount of variation between tumor types. For example, the cancer types most frequently mutated are T-cell acute lymphoblastic leukaemia (T-ALL) and cholangiocarcinoma with 31% and 35% of cases harbouring a mutation in *FBXW7* respectively [1]. In contrast, prostate cancers has a 6% mutation rate [63].

The FBXW7 protein contains an F-box protein interaction domain that interacts directly with SKP1 (S-phase kinase associated protein 1) and is responsible for the recruitment of ubiquitination substrates to the SCF [62]. It also contains eight WD40 repeats that are used to interact with its phosphorylated protein substrates at the CPD domain [46], and a D domain that is used for the dimerization of FBXW7 [129]. The third and fourth WD repeat domains of FBXW7 contain 3 conserved arginine residues that interact with the phosphorylated substrates, while the remaining repeats are responsible for substrate binding [46, 86].

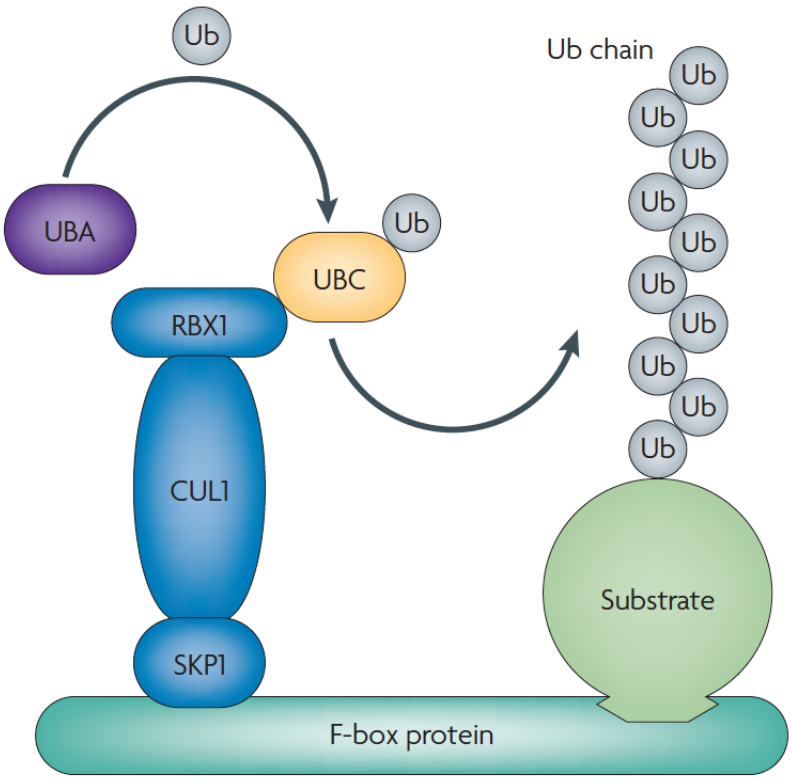

Figure 1 | **Schematic of an E3 SCF (complex of SKP1, CUL1 and F-box protein) ubiquitin ligase.** The SKP1 (S-phase kinase-associated protein 1)–CUL1 (cullin 1)–RBX1 (RING box 1) core complex recruits substrates through interchangeable substrate-specific F-box proteins. RBX1 binds to the E2 ubiquitin-conjugating enzyme (UBC) that was previously charged with ubiquitin (Ub) by an E1 ubiquitin-activating enzyme (UBA). Polyubiquitylated substrates are then targeted for destruction by the proteasome.

Figure 2.1: A schematic diagram of the SCF protein complex reprinted with permission from Nature Reviews Genetics (Appendix C.1) [130].

Given that FBXW7 is responsible for the degradation of several oncogenes, it is not surprising that targeting FBXW7 and it's substrates could be a viable approach to treating cancers. Targeting FBXW7 directly is one option given that a large portion of tumors maintain *FBXW7* expression. However, in cancers where *FBXW7* is lost, a better approach might be to target the oncogenic substrates of FBXW7. This has already been achieved for two substrates of FBXW7: in human T-ALL Notch has been targeted using γ-secretase inhibitors, and in murine sarcomas, MYC has been targeted using γ-secretase inhibitors as well [42, 57]. Another potential avenue for targeting cancers through loss of *FBXW7* is to evaluate SL interactions with *FBXW7* and use those genes as potential drug targets. This strategy would be particularly useful for cancers with low expression of *FBXW7* since it's SL partners can be targeted with drugs on a patient by patient basis.

2.2 Yeast as a model organism

Model species are often extremely useful starting points for research. In the case of yeast, there are several studies that support its utility and application to human research, especially cancer research [11, 43, 75, 91, 92, 95, 97, 104]. Hartwell was the first to discover genes involved in the yeast cell cycle [49, 50, 95]. These cell division cycle (CDC) genes in yeast have been studied extensively in a human cancer context [8, 17, 71, 99], and mutations in these genes in humans have been shown to be involved in cancer [95]. In addition to having genes that are relevant to studying human cancer, yeast is also easily cultured in a laboratory and has a relatively small genome [41]. Furthermore, there are currently many research groups focusing on yeast resulting in large amounts of data that can be mined and applied to human cancer research [11, 43, 75, 91, 92, 95, 97, 104].

2.2.1 Ortholog mapping

Prior to discussing the ways of mapping yeast genes to human genes, it is important to identify and define the following key evolutionary terms: homologs, orthologs, and paralogs. Homologs are genes with a common evolutionary origin but not necessarily the same function and can be divided into orthologs and paralogs. Orthologs are genes with a common ancestor

that are separated by speciation and have kept the same function over time. In contrast, paralogs are genes with different functions in the same genome related by duplication. There are two main ways that ortholog mapping is conducted *in silico*: sequence similarity (for example using a BLAST-based approach (basic local alignment search tool) [56, 70, 109]) and a phylogeny-based approach [55, 96, 100, 141]. The BLAST-based approach involves pairwise sequence comparison and is a fast approach for identifying potential orthologs. In contrast, the phylogeny-based approaches compare gene trees to species trees to identify gene duplication events and predict orthoogy. Furthermore, phylogeny-based approaches are more time- and resource-intensive but are useful in identifying more fine-grained ortholog relationships. InParanoid was built using a sequence similarity approach and has an easy-to-use interface.

2.3 Synthetic lethality

There are many different genetic interactions that researchers can exploit in order to identify potential genes that would act as drug targets for cancer therapeutics. Genetic interactions are the phenomenon where two or more gene products interact with one another that results in a deviation of the expected phenotype [78]. Two such genetic interactions are synthetic lethality and synthetic dosage lethality. A SL interaction involves a pair of genes (geneA and geneB) where inhibition of either geneA or geneB individually has no effect on cell viability, but the inhibition of both geneA and geneB causes cell death. In contrast, a synthetic dosage lethal (SDL) interaction involves a pair of genes (geneA and geneB) where over-expression of either geneA or geneB individually has no effect on cell viability, but the inhibition of geneA when geneB is over-expressed causes cell death (Figure 2.1). The concept of synthetic lethality was first described in 1922 by Calvin Bridges [15]; however, recent applications to cancer and the discovery of complex SL interactions (such as SL triples and quadruples [74]: SL interactions where all three or four genes need to be down-regulated for the lethal phenotype) are more recent applications of the concept. There are three proposed mechanisms of SL interactions: (1) each gene is involved in parallel redundant cellular pathways, (2) genes are members of distinct cellular pathways that generate the same product, and (3) the genes are

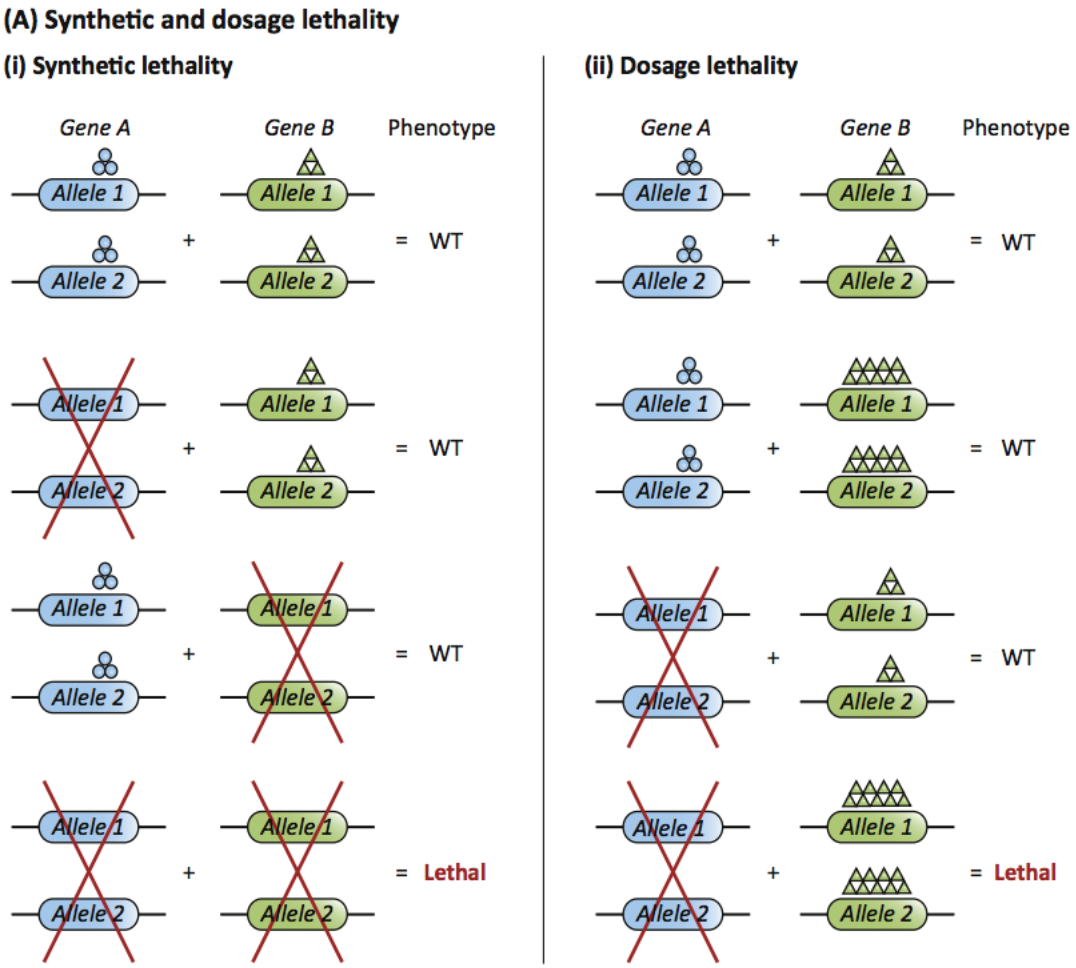

Figure 2.2: A schematic diagram of SL and SDL reprinted with permission from Cell Press (Appendix C.2) [89]. (i) represents a schematic of SL and (ii) is a schematic representation of SDL.

translated into different subunits of the same protein complex (Figure 2.2) [34].

Synthetic lethality has a wide array of applications in microbial, plant, animal, and human research. The most effective means of accurately identifying SL interactions is through *in vitro* assays that knock down both genes and monitor cell survival. This is often done *in vitro* and *in vivo* using CRISPR-Cas9 [102] or RNA interference [32]. Despite similar methods for identifying SL interactions, applications in microbes, plants, and animals (including *Drosophila melanogaster* and *Caenorhabditis elegans*) are different from cancer applications in humans. For example, in bacteria SL interactions have been evaluated to determine essential plasticity (the SL interaction works as a backup mechanism, e.g. Cell Envelope

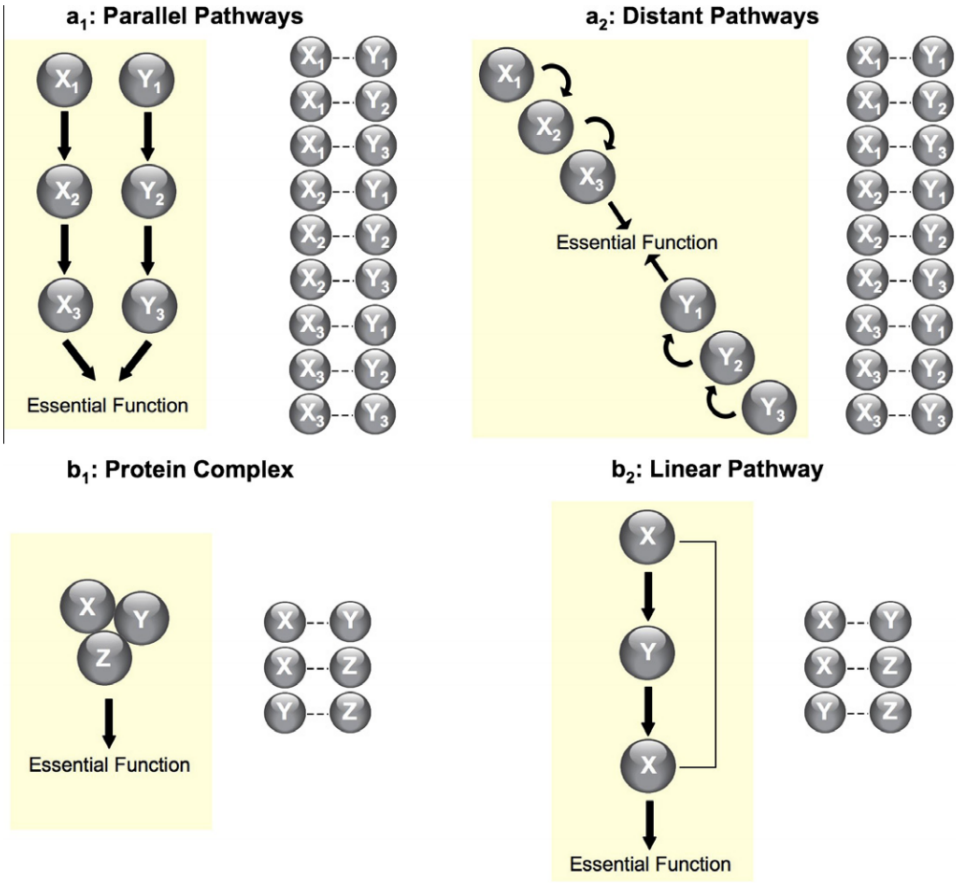

Fig. 1 – Different mechanisms leading to synthetic lethality. The possible SL gene pairs are reported on the right side of each panel. Synthetic lethality can arise form the absence of two genes acting in redundant parallel pathways (a_1) or distant pathways (a_2). Alternatively, it can originate from the lack of two subunits of an essential protein complex (b_1) or two proteins of the same essential pathway (b_2).

Figure 2.3: A schematic diagram of mechanisms of SL reprinted with permission from European Journal of Cancer (Appendix C.3) [34].

Biosynthesis as an essential backup for Membrane Lipid Metabolism) and essential redundancy (the SL interaction works as a parallel mechanism - shown by SL interactions affect a single function or pathway e.g. apoptosis) in metabolic networks [44]. In other microbial studies, SL interactions are used to identify gene functions in metabolic pathways [117], predict flux distribution and metabolic function for increased production of biofuels [51], analyze metabolites in known pathogens [40], and discover antibiotic combinations [3]. Furthermore, a large number of studies have focused on SL interactions in yeast, specifically *Saccharomyces cerevisiae*. These studies include mining protein-protein interaction networks for SL interactions [88], screening for genome instability [139], flux balance analysis (a mathematical model for simulating metabolism) to identify SL interactions relevant to metabolic pathways [47], identifying genes required for chromosomal segregation [77], identifying complex molecular interactions through SL interactions in multi-protein complexes [65], gene function prediction [138], and examining the total set of SL interactions in the yeast genome [22, 23, 84].

Although *in vitro* assays are important for validating SL interactions, they can be time-consuming, expensive, and labour-intensive, especially if the genome of the organism being studied is large in size. For example, studying SL interactions *in vitro* in human cancers requires extensive cell line work and the generation of millions of double-mutant cell lines in order to evaluate all possible SL interactions. Given approximately 20,000 genes in the human genome, there are almost 200,000,000 genetic interactions in an exhaustive *in vitro* screen. To that effect, identifying higher order SL triples or quadruples *in vitro* becomes increasingly more expensive and time-consuming as the dimensionality of the problem increases [74]. As a result, researchers are beginning to exploit SL interactions in model organisms (such as yeast [22, 23, 84]) to identify promising SL candidate drug targets. These approaches are more time- and cost-effective since they reduce the number of potential SL interactions to a feasible set that can be evaluated *in vitro* in a reasonable amount of time. Additionally, computational studies are working on developing predictive models for synthetic lethality that will serve as a form of dimensional reduction, which means that *in vitro* resources can focus on validating SL interactions that have already been computationally predicted [27, 58, 74, 105, 114]. Therefore, it is not surprising that there is an increased interest in computational approaches to identifying SL interactions in humans that make use of previously identified SL interactions

in model species.

2.4 Computational approaches for identifying SL interactions

Often, if geneA is mutated, down-regulated, or not expressed in cancer and it is in a SL interaction with geneB, then geneB becomes a potential target for novel or existing drugs. This type of targeted drug-gene interaction can reduce the side effects of the drug, increase the efficiency of treatment, and increase the comfort of the patient during treatment. It can also prevent patients from being treated with drugs that will not be as effective for their cancer, reducing the cost and length of patient treatment and potentially preventing negative side effects like liver damage from excessive (or unnecessary) chemotherapy. Computational approaches to synthetic lethality in cancer are increasing in popularity using existing cell line gene expression data, patient survival and mutation data, and previously experimentally validated SL interaction data from model organisms (e.g. yeast) [27, 58, 74, 94, 114].

Some programs are designed just for the purpose of identifying SL interactions in microbes, such as Fast-SL [94]. Fast-SL takes a linear programming approach to flux balance analysis (FBA). FBA uses metabolic models to analyze fluxes in a network and analyzes gene enrichment analysis, allowing for gene essentiality analysis and potential identification of SL interactions [120]. Fast-SL was used to analyze *Escherichia coli*, *Salmonella enterica* Typhimurium and *Mycobacterium tuberculosis* for SL interactions. It not only identified SL gene deletions in all three species but also identified novel SL triples. This approach to SL identification has applications in the identification of novel antibiotic targets and can help researchers explore complex genetic interactions in pathogens.

In addition to studies that are focused solely on bacteria and yeast, there are also several studies that identify SL interactions in yeast with the intention of applying them to human cancer, or use existing experimentally validated yeast SL interactions to identify cancer relevant SL interactions. Using yeast and other species as model organisms for studying SL interactions in humans can be extremely valuable, resource efficient, and help reduce the sample size for *in vitro* and *in vivo* testing. For example, Meta-SL is a computational

approach to identify SL gene pairs in yeast combining information from 17 genomic and proteomic features with the results of several classification models [133]. Upon the identification of SL interactions in yeast, ortholog mapping from yeast to humans revealed a set of cancer relevant SL interactions to study further.

Other computational studies use previously identified yeast SL interactions to identify potential SL interactions in human cancer [27]. Deshpande *et al.* use existing yeast SL interaction data from The Cell Map [22], ortholog mapping from yeast to humans, and cancer mutation data in order to identify potential cancer relevant SL interactions. This study identified 1,522 SL interactions relevant to cancer and tested SL interactions between *SMARCB1* and *PSMA4*, and *ASPSCR1* and *PSMC2* as proof of concept. More than 500 SL interactions containing at least one gene that is known to be associated with cancer were identified in this study.

In contrast, Srivas *et al.* identified a list of human cancer genes, mapped them to yeast orthologs and their SL interactions, and tested the resulting SL interactions in human cell lines [114]. This strategy is arguably more cancer directed as it starts with a list of known human tumor suppressor genes (TSG) and applies existing yeast genetic interaction data. Though the results are highly relevant to human cancer, they are extremely focused on known TSGs and there is the potential for the exclusion of SL interactions that might involve a previously unidentified human TSG. Despite their differences, both of these research endeavours focus on previously identified genes associated with cancer.

In contrast, the identification of SL interactions in humans without the use of a model organism is complicated by the size of the genome, which greatly increases the number of possible SL interactions. As a result, a number of specialized programs have been implemented for the identification of SL interactions specifically in human cancer. One of the more recent research efforts by Matlak and Szczurek [74] introduces SurvLRT, a program that analyzes cancer patient data using a statistical likelihood ratio test in order to identify SL gene pairs and triples in humans. SurvLRT is the first computational approach that identifies biomarkers for SL drug therapy, and was successful in identifying *TP53BP1* as a biomarker for the well established *PARP-BRCA1* SL gene pair. What's more, SurvLRT identified a novel biomarker for the same SL interaction that has a higher number of deletions

in cancer patients than *TP53BP1*, opening new research avenues for personalized medicine in cancer therapy.

Another computational pipeline for the identification of SL and SDL interactions in human cancer is DAISY (data mining synthetic lethality identification pipeline) [58]. DAISY makes use of multiple cancer patient and cancer cell line datasets and uses three statistical inference strategies to predict SL interactions in cancer. The first inference strategy, genomic survival of the fittest (SoF), analyzes somatic copy number alterations (SCNA) and mutation profiles for gene pairs where gene B has higher SCNA values and where geneA is inactive. The next strategy, shRNA-based functional examination, uses gene essentiality profiles to determine if, for each gene pair, geneB has lower shRNA expression in samples where geneA is inactive. Finally, DAISY evaluates pairwise gene co-expression using gene expression profiles to identify gene pairs whose expression is positively correlated. Gene pairs that are statistically significant in all three of the inference strategies are reported as positive SL interactions in human cancer.

Unfortunately, because DAISY uses a small number of mutations and shRNA data from existing cell lines, it misses all SL interactions that are false negatives in shRNA screens due to a lack of representation in existing cancer cell lines or due to incomplete knockdown. To counter these shortcomings, Sinha *et al.* [105] developed MiSL (mining synthetic lethals) to identify cancer specific SL interactions in specific cancer types. The MiSL algorithm is based on Boolean implications mined from pan-cancer datasets. Because MiSL takes into account all types of mutations, compared to DAISY which only takes into account frame shift and non-sense mutations, MiSL out performs DAISY by identifying several novel SL interactions that DAISY can't identify. This does not mean that MiSL is perfect; rather, it shows that research is constantly being completed to improve computational approaches for SL identification in humans.

Since these computational methods rely on the use of existing datasets, there is still a need to validate their conclusions *in vitro* (and sometimes *in vivo*) to ensure that the computational method is accurate. However, with an increasing number of SL interactions identified through cell line testing and a database for synthetic lethal interactions (SynLethDB [45]), it is now possible to validate *in silico* approaches by comparing the results to SL interactions that

have been previously identified in the lab and by other SL computational approaches. This may pose challenges though as a novel computational method of identifying SL interactions in humans might have no SL interactions in common with previous research and the predicted SL interactions have to be validated through *in vitro* and *in vivo* studies. However, results that have overlap with existing computational methods can be partially validated by analyzing those interactions. *In vitro* and *in vivo* studies can then provide additional support for the interactions that overlap with existing studies.

Unfortunately, there only a few databases that contain human SL interaction data from multiple studies. The most well known is the SynLethDB [45], which contains SL interactions from large scale computational studies, SL interactions recovered from text mining, and computationally derived SL interactions from the DECIPHER database [36]. Unfortunately, there are errors in this database (see Results and Discussion), which result in a loss of confidence in the text mining entries and reduction in the number of SL interactions available. However, SynLethDB is a starting point for comparing known SL interactions to those identified in novel computational studies.

This thesis combines previously identified yeast genetic interaction data with yeast-human orthology data to develop a novel network of human SL interactions that can be computationally verified using existing studies. As mentioned earlier, Deshpande *et al.* [27] used existing SL interaction data from yeast to identify SL interactions in human cancer (this topic expanded on in section 6.1). The research described here is based on their approach; however, updated databases and patient gene expression data from The Cancer Genome Atlas (TCGA) is used to generate a breast cancer specific set of SL interactions in humans. Using this information in conjunction with SynLethDB entries, we show that the network of predicted SL interactions in humans is not the result of random chance and further identify 32 druggable gene targets specific to breast cancer.

2.5 Data sources for computational studies

In order to use a computational approach to identify human SL interactions previous data and experimental results were necessary to begin the analyses. The main sources of data for

the work presented in this thesis were: The Cell Map [123], the TCGA database (`https://cancergenome.nih.gov/`), SynLethDB [45], and the InParanoid database [109]. In addition to these main data sources several additional data sources were used such as: cancerRXgene database [136], the cancer cell line encyclopedia (CCLE) [7], the Ensembl database [141], and the work of Deshpande *et al.* [27] in order to curate, compare and analyze the data in this thesis.

2.5.1 The Cell Map

As previously discussed, yeast is a good model organism for studying cancer in humans (Section 2.2). The starting dataset for the generation of the HYGIN network was The Cell Map [123]. Cell Map is a current dataset of previously biologically validated yeast genetic interactions that was last updated May 2016. The dataset covers close to 90% of yeast genes and contains approximately 550,000 negative and 350,000 positive yeast genetic interactions. In the Cell Map dataset, deletion query mutant strains were screened against deletion mutant arrays in the search for genetic interactions. Interactions were scored based on an ϵ value (a score for genetic interactions[1]) and statistical significance. Using the data cut-off values for P-value and ϵ as described in the work by Deshpande *et al.* [27], a subset of the Cell Map data was used to generate the HYGIN network.

2.5.2 InParanoid

Orthologous mapping of yeast to human genes used the InParanoid database of orthologs [109]. As mentioned previously, the InParanoid database contains 273 species, 37,128 species pairs, and 2,999,062 orthologous proteins including 2,041 orthologs between yeast and humans [109]. InParanoid uses sequence similarity implemented through a BLAST-based approach to ortholog mapping and accounts for one-to-one and one-to-many orthologs between pairs of species. InParanoid uses bootstrap confidence values to assign a confidence score that the seed used for the comparison and it's ortholog are true orthologs. InParanoid version 8 is the

[1]The specific details for how ϵ was calculated were not provided in the original manuscript, nor were explicit details given in the original Science paper [122].

most current version [109], and it is updated regularly to include new genetic information for identifying orthologs.

2.5.3 The synthetic lethal database

SynLethDB is a curated database that contains SL gene pairs from other databases, biochemical results, text mining, and computational predictions [45]. In addition to 19,952 human SL interactions, the SynLethDB contains SL interactions for *Saccharomyces cerevisiae* (13,241), *Drosophila melanogaster* (423), *Mus musculus* (366), and *Caenorhabditis elegans* (107). Two of the major contributors to the human SL interactions in the SynLethDB are a computational study, DAISY (discussed in Section 2.3) [58], and SL interactions that were identified through an analysis of the DECIPHER database [36]. The remaining major contributors to the SynLethDB are other smaller *in vitro* analyses and SL interactions identified through a text-mining algorithm. The text mining approach that Gou *et al.* used for SynLethDB involved three major steps: search, train, and extract. During the search step, the titles of the articles in the PubMed database were searched for the terms "synthetic lethal" and "synthetic lethality". The abstracts of the 331 articles that were identified to contain these terms in their titles were used in to train the literature ranking tool, MedlineRanker [37]. MedlineRanker uses a defined training set (e.g. the previously mentioned 331 abstracts) to rank the literature in a database according to how relevant they are to synthetic lethality. Gou *et al.* chose to use the top 1,000 articles from the past 10 years in their search for SL interactions from previous literature. The third step in their text mining procedure was to use PESCADOR (Platform for Exploration of Significant Concepts AssociateD to co-Occurrence Relationships) [6], an online tool for identifying biointeractions from articles in the PubMed database. Based on the information from MedlineRanker, PESCADOR used keywords to identify SL interactions in the literature. Although the text mining portion of the SynLethDB contained several errors (see Section 5.2 for greater detail and examples), text mining approaches are a valuable means of populating databases and consolidating information for *in silico* studies provided that checks and balances are in place. The authors report that they will address the errors in the upcoming version of the database.

2.5.4 The cancer genome atlas

The cancer genome atlas is currently one of the most up-to-date comprehensive publicly available cancer genetics databases (`https://cancergenome.nih.gov/`). In addition to cancer gene expression data for more than 10,000 cancer samples across 33 cancer types, the TCGA also contains mutation data, patient data, and raw sequencing data. Additionally, the TCGA contains non-cancerous gene expression data for some cancer patients, including 114 normal breast tissue samples from 114 breast cancer patients. This paired data, i.e. gene expression data from normal tissue and cancerous tissue from the same patient, is extremely valuable for researchers. Specifically, it gives researchers the ability to evaluate differential gene expression in cancer versus normal tissue while accounting for baseline gene expression in individual patients.

Chapter 3

Research Hypothesis

Although the principle of using bioinformatics techniques for the identification of SL interactions *in silico* is not new, there are ways that we can improve upon previous techniques and approaches to better identify SL interactions in breast cancer. With a constant increase in the amount of patient genomic data and an ever-increasing number of cancer cell lines (and genomic data for these cell lines), novel practical approaches are needed for the analysis of this data. Furthermore, there are also a number of studies that have started with experimentally validated SL interactions in non-human model organisms and used this data as a spring board for the identification of SL interactions in human cancer. This thesis describes an improved approach using yeast orthologs to develop a humanized genetic interaction network in a cancer independent context.

This thesis used yeast genetic interaction data from The Cell Map [23] and cancer gene expression data from the TCGA to identify breast cancer specific SL interactions. We hypothesize that the creation of an improved and updated humanized yeast genetic interaction network based on yeast-human orthologs and the application of breast cancer gene expression data will allow us to identify genes for targeted breast cancer therapeutics. The pursuit of this hypothesis was broken down into the following goals:

1. Curate the existing yeast genetic interaction network from The Cell Map, and convert it to a humanized genetic interaction network that uses gene identifiers that can stand the test of time with minimal changes.

2. Identify the set of genes that are down-regulated in breast cancer patients using TCGA data.

3. Map the set of down-regulated genes onto the humanized yeast genetic interaction

network to generate a breast cancer specific subnetwork.

4. Identify genes that have SL interactions with multiple cancer related genes that are down-regulated in breast cancer.

5. Identify potential novel drugs for target genes using the drug-gene interaction database.

The goal of this project is to generate a humanized yeast genetic interaction network (HYGIN) based on human orthologs in yeast in a cancer independent context, apply cancer gene expression data, and identify novel SL gene pairs for targeted breast cancer therapeutics.

Chapter 4

Data and Methodology

Using yeast genetic interaction data as the basis for identifying SL interactions in humans is not a novel concept. Previously, the work of Deshpande *et al.* used yeast genetic interaction data to generate a cancer-independent human SL interaction network [27]. However, their network used all yeast-human orthologs including instances of one-to-many and many-to-many mapping. Using an updated version of the yeast interaction dataset [23], and working to improve on the strategy of Deshpande *et al.*, this thesis generates a stringent humanized yeast genetic interaction network that uses strict one-to-one yeast-human ortholog mapping. We then use breast cancer data from the TCGA and generate a breast cancer specific subnetwork that contains potential gene targets for breast cancer therapeutics.

4.1 Generating the humanized genetic interaction network

In order to generate HYGIN, experimentally validated yeast interactions from the May 2016 release of The Cell Map [23] were used. The yeast network contains quantitative genetic interactions for all gene pairs in *Saccharomyces cerevisiae* and is the result of double mutant arrays conducted in yeast that represent nearly all of the SL interactions in the yeast genome.

The yeast interaction data contains approximately 550,000 negative genetic interactions (SL interactions) between approximately 90% of the genes in the yeast genome [23]. In order to generate a humanized network, the yeast network was translated in three stages: yeast gene name to yeast protein, yeast protein to human orthologous protein, and finally human protein to human gene name. The UniProt database [2] was used for the first and last stages

and InParanoid Version 8.0 [109], an online tool for identifying orthologs between two species, was used to identify the human orthologs of the yeast proteins in stage two. Strict one-to-one mapping with InParanoid was used for the network to prevent ambiguities in the translation process (Supplementary Table B.1).

For example, The Cell Map contains the synthetic lethal interaction between the yeast genes *RPN1* and *MET30*. The names of these two genes were converted to yeast protein accession numbers (P38764 and P39014 respectively). InParanoid ortholog mapping then mapped the yeast protein accession numbers to human protein accession numbers (Q13200 and Q969H0 respectively). Finally, the human protein accession numbers were mapped to the corresponding human genes *PSMD2* and *FBXW7*, and a SL interaction between these genes was added to the HYGIN network. Supplementary Table B.2 lists tall the interactions in HYGIN.

Performing the mapping and filtering steps is tedious and error prone. Therefore the process of generating HYGIN was automated and performed computationally using a combination of bash (UNIX shell) and Python scripts. The network information was imported into Cytoscape [103] for visualization and analysis. The resulting network has 1,009 nodes (human genes) and 10,419 edges (proposed SL interactions between human genes). A summary of this process can be found in Figure 4.1.

4.2 Creating the breast cancer specific subnetwork

HYGIN was developed in a cancer independent manner in order to avoid certain biases such as starting with known oncogenes or TSGs, or limiting yeast ortholog mapping to only known cancer associated genes in humans. As a result, HYGIN is disease independent and the SL interactions in the network could be used for multiple different studies. However, our research hypothesis involved identifying SL interactions that are relevant to breast cancer. As a result, breast cancer data needed to be incorporated into HYGIN in order to identify potential gene targets for breast cancer therapeutics. This was accomplished through the integration of TCGA breast cancer data with HYGIN and the generation of a breast cancer specific subnetwork. The TCGA contains gene expression data for both normal (114 samples)

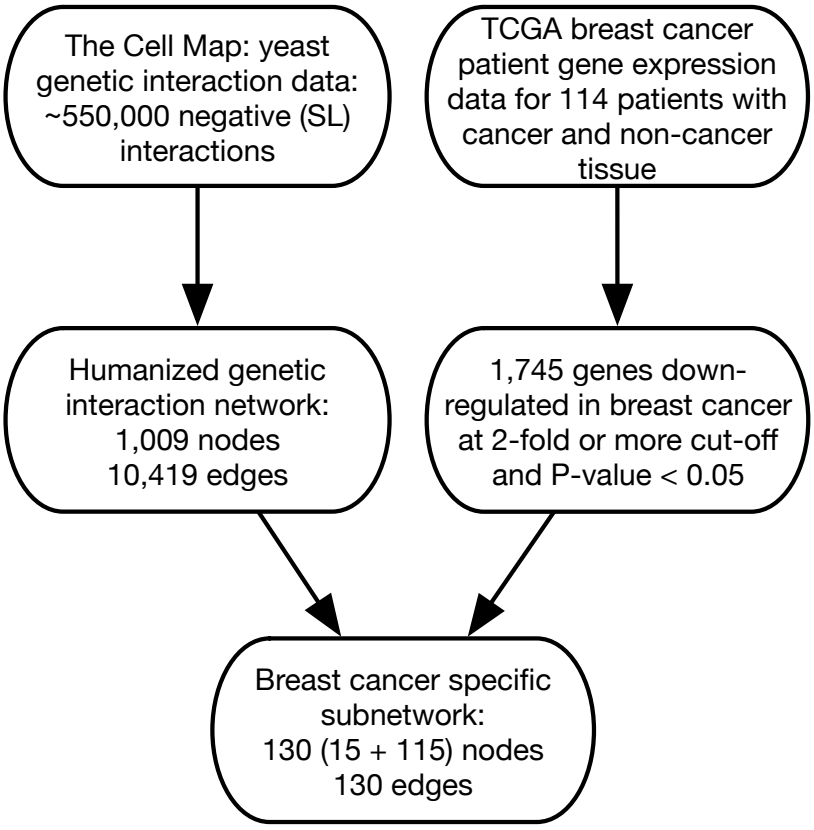

Figure 4.1: Flow diagram showing the flow of data and method used to generate the breast cancer specific subnetwork.

and cancerous (1,104 samples) tissue.

Some studies use all available data as a means of reaching statistically significant results, or as a means of completing an exhaustive analysis [58, 106], for example, using pooled normal tissue data and comparing it to pooled cancers tissue data as a means of statistically evaluating changes in gene expression. However, we used a more stringent methodology and only analyzed TCGA patient data for which there was gene expression data for both breast cancer tissue and normal breast tissue from the same patient. This increases the confidence in determining gene up- and down-regulation as it helps eliminate false positive results that occur due to differences in basal gene expression between individuals. By comparing up- and down-regulation of genes between two like-tissue samples from the same patient, changes in expression are more reliable than pooling cancer samples and comparing them to pooled normal samples. Although this strategy is very stringent, it is also one of the strengths of our gene expression analysis.

Gene expression data was downloaded from the TCGA database (`https://cancergenome.nih.gov/`). Gene expression data for cancerous tissue and normal tissue in the same patient was available for 114 patients: level-3 HiSeq RSEM gene-normalized RNA-seq data was obtained for 1,104 cancerous and 114 normal samples for 20,530 genes. However, some genes had zero values for one or more patients as a result of either there being no transcripts sequenced, or transcripts missing for a particular gene. Consequently, different methods were attempted to resolve these issues, some of which were not included in the final analysis.

4.2.1 Initial analysis

The initial data analysis workflow for identifying genes down-regulated in breast cancer removed genes with some (84 or more) expression values of zero. The TCGA dataset for breast cancer contains normal and cancerous gene expression data for 20,531 genes in both cancerous and normal tissue across 114 patients. Since the TCGA gene expression datasets for breast cancer are incomplete (i.e. there are numerous genes that do not have 114 expression values for all patients), there were instances of genes with zero values for either cancerous and/or normal samples certain from patients. There were 6,575 genes that had gene expression values of zero for between 1 and 114 patients from cancerous and/or normal

tissue datasets. As a preliminary step, approximately 1,000 genes with zero values for all 114 patients were removed from the analysis entirely due to the lack of data. However, some genes still had zero values for the majority of patients, which would invalidate down-stream statistical analyses. As a result, in order to have sufficient data to complete valid statistical tests (whether parametric or non-parametric), a minimum of 30 non-zero gene expression values were required for each gene. Therefore, 18,654 genes out of 20,531 genes total with at least 30 non-zero values were identified for further analysis in the initial analysis method.

When using statistics to analyze large datasets, it is important to use an appropriate test that reflects the normality of the data. The Shapiro-Wilk test for normality tests datasets to determine whether or not they follow a normal distribution. In order to maintain stringency in our analysis, the Shapiro-Wilk test was conducted on the expression values of the 18,654 genes that had at least 30 non-zero values. The results of this test showed that the gene expression data did not have a normal distribution even though the sample size would have been large enough to make the assumption of normality [28]. Therefore, a non-parametric test was used to analyze this data as it is a more appropriate statistical method for the data.

In spite of the above method of maintaining some zero values in the dataset, by evaluating samples with 30 to 114 paired values per gene (where there is a non-zero value for both cancer and normal tissue), there was the potential for misrepresentation in some genes compared to others, which could lead to potential false positive results. For example, geneA could be found to be down-regulated in breast cancer with a sample size of 30, but in reality if we had genetic data from 114 patients we would see no statistical change in gene expression. As a result, this workflow was not used to generate the breast cancer specific subnetwork.

4.2.2 Final analysis

To improve upon the initial analysis, and to ensure that the results moving forward were statistically sound, any gene for which there was one or more expression value of zero in either cancerous or normal tissue were removed in the final version of the analysis. For example, if there is a zero value in the cancer data set for geneA, then that gene is removed from the analysis regardless of whether it has a zero value in the dataset for normal tissue. This filtering procedure resulted in analyzing data for 13,938 genes out of the 20,531 genes

in the TCGA breast cancer dataset.

Once zero values had been accounted for and dealt with accordingly, the Shapiro-Wilk test was conducted again on the remaining 13,938 genes. The Shapiro-Wilk test showed that although more than 30 samples were present for each gene, the expression values were not normal and a non-parametric statistical test was required. The Wilcoxon signed-rank test was selected as the non-parametric statistical test for the paired data to determine if the median gene expression between the cancer sample and the normal sample was statistically different ($P < 0.05$). Since greater numbers of statistical inferences made from a dataset increases the chances of an error in any of those inferences, it was imperative to account for multiple hypotheses.

4.2.3 Multiple hypothesis testing

Two types of multiple hypothesis corrections were considered for the data analysis, the Bonferroni correction and the Benjamini-Hochberg procedure. The Bonferroni correction corrects for family-wise error rate (FWER), whereas the Benjamini-Hochberg procedure corrects for false discovery rate (FDR)[118].

FWER represents the probability of making a Type I error (false discovery) when testing multiple hypotheses using the same data and reduces the probability of making a type I error with respect to the results returned as positive compared to the original P-value. In contrast, FDR conceptualizes the rate of Type I errors when conducting multiple comparisons. The Bonferroni correction is one of the most strict corrections for multiple hypotheses. Adjusted P-values are calculated by dividing each critical value by the total number of hypotheses (n) being tested (e.g. P-value/n). This procedure affects all critical values equally, which can lead to false negative results because it is so strict. Also the Bonferroni corrections results in a group of hypotheses where α (the allowable percentage of false positive results e.g. 0.05) results are expected to be false positives. Furthermore, the more tests (or the larger n), the greater the effect the Bonferroni correction will have on P-values. In contrast, the Benjamini-Hochberg procedure affects P-values based on their rank (e.g. (P-value's rank/n) * α) where α is the probability of making a Type I error). FDR procedures, such as the Benjamini-Hochberg procedure, are less stringent when it comes to false positives in the data; however,

they have greater statistical power. As a result, for our Wilcoxon signed-rank test on the TCGA data we used the Benjamini-Hochberg procedure, choosing increased statistical power at the cost of potential false positives in the results. In contrast, the Bonferroni correction was used for the Shapiro-Wilk tests for normality on the cancer and non-cancer datasets. It was selected because it is a more stringent statistical test and we wanted to ensure that normality was not being incorrectly assumed.

4.2.4 Determining gene down-regulation in breast cancer

In order to determine if a gene was down-regulated in breast cancer, two analyses had to converge: the Wilcoxon signed-rank test results and the evaluation of gene expression ratio in breast cancer. To determine the fold change of gene expression between the cancerous and normal tissue, the ratio of gene expression was calculated (cancer/non-cancer) and the log-base-2 of the ratio was used as the fold change in gene expression in breast cancer. Once the fold change was calculated and Wilcoxon signed-rank test P-values were corrected for multiple hypotheses, a cut-off value was needed to determine which genes were statistically down-regulated in breast cancer.

We evaluated several potential cut-off values: 2-fold, 3-fold, 4-fold, 5-fold, 8-fold, and 16-fold representing biological significance and one standard deviation representing statistical significance. Though all of these cut-off values are technically valid, it was appropriate to select the cut-off value that most accurately addresses the following question: which genes are down-regulated in breast cancer tissue compared to normal tissue? Since we are asking a biological question, a cut-off that represents an accurate biological significance threshold should be used. As the fold change increases the results become more stringent and the number of significant genes decreases. Biologically a higher fold change means that genes have a greater change in expression when comparing cancerous to normal tissue. However, the aim was to identify genes that are down-regulated, not genes that have large differential expression in breast cancer. As a result, a lower fold-change threshold makes more biological sense. Since a 2-fold cut-off represents instances where a gene in breast cancer has half of the expression observed in healthy breast tissue, it was determined that this cut-off was an accurate representation of the biological question. Consequently, genes that were down-

regulated at a 2-fold or more cut-off in breast cancer and had a P-value (after adjustment) < 0.05 were considered to be down-regulated in breast cancer.

4.2.5 Generating the breast cancer specific subnetwork

To give the SL interactions in HYGIN context in cancer, the results from analyzing the TCGA breast cancer data were used to generate a breast cancer specific subnetwork of HYGIN. The breast cancer subnetwork was generated by extracting the genes that were down-regulated in breast cancer and all of their SL interactions from HYGIN. This subnetwork contains the potential 130 SL interactions (edges) that are specific to breast cancer between 130 genes (nodes) total; 15 genes that are down-regulated in breast cancer and 115 genes that have SL interactions with them.

4.3 Validating HYGIN

HYGIN was validated *in silico* using the Synthetic Lethal Database (SynLethDB; July 12, 2017) [45] and a series of random networks. The SL interactions in the SynLethDB are a combination of computationally predicted SL interactions, and SL interactions that have been identified through large- or small-scale *in vitro* assays. Unfortunately, the text mining algorithm for SynLethDB did not accurately identify SL interactions from the literature. As a result they were all excluded from our validation procedure. Using the remaining information in the database, we identified 11 SL interactions from HYGIN that have been identified by previous studies. Eight of these interactions were computationally identified by DAISY [58], and 3 were identified by SynLethDB through analysis of data in DECIPHER [36].

To determine if the 11 interactions identified by previous studies were statistically significant or if they were the result of random chance, we generated 100,000 iterations of a random network that mimics the original yeast interaction network. By generating multiple random networks we can calculate the number of times that there were 11 or more SL interactions in common with HYGIN that were the result of random chance. The random networks contained the same number of nodes as there were yeast genes (5,587) and the same number of edges as there were yeast SL interactions (132,312) in the yeast SL interaction network. Al-

though there were approximately 550,000 negative genetic interactions in The Cell Map data, only statistically significant entries were used for the generation of HYGIN and subsequent analyses ($P < 0.05$ and $\epsilon < -0.2$, where ϵ is the genetic interaction score used by Costanzo *et al.* [23]). They were converted to a humanized genetic interaction network using the same procedure as for HYGIN, and evaluated for SL interactions in common with the SynLethDB. Random networks and comparison were completed using the Python library NetworkX.

4.4 Verifying one-to-one ortholog mapping of FBXW7

FBXW7 was selected as a target of interest for future analysis not only because it is a known tumor suppressor, but because it is highly connected in the breast cancer subnetwork. With 32 potential SL interactions that can be exploited as therapeutic options, FBXW7 is an ideal candidate for further testing *in silico*, *in vitro*, and *in vivo*. Since one of the major motivations for using one-to-one yeast-human ortholog mapping was the lack thereof in the previous work by Deshpande *et al.*, it was important to verify the one-to-one mapping for the gene of interest. To confirm that there were no additional yeast-human orthologs of FBXW7, the InParanoid database [109] was searched twice: the human genome was searched for the protein MET30 (the yeast protein that is orthologous to FBXW7 in humans) to ensure that there was only one ortholog for the yeast MET30 protein, and for CDC4 (a common synonym for FBXW7 in humans) to ensure that there were no additional orthologs for FBXW7 in the database under its alias CDC4.

4.5 Gene expression in other cancers

A recent study showed that on average 43% of cancers from a specific tissue type were more similar to tumors from a different anatomical site than they were to tumors of the same tissue of origin [24]. Although generally cancer is diagnosed and treated based on the tissue or origin, there is more and more evidence that supports the theory that genetically cancers from different tissues can be more alike than not [53, 61]. As a result, the 15 genes that were in HYGIN and identified to be down-regulated in breast cancer were also analyzed in the

24 types of cancer in the TCGA database. For each of these 24 cancer types, pooled gene expression for cancer tissue was compared to pooled gene expression for normal tissue. Since we were evaluating these cancers for down-regulation, only cancer types where more than 7 of the 15 genes had lower expression in cancer than in normal tissue were reported.

4.6 Drug data analysis

In an attempt to validate the SL interactions of FBXW7 *in silico* we used cancer cell line drug sensitivity data and cancer cell line gene expression data. We performed an *in silico* drug sensitivity assay using data from the cancerRXgene database which contains drug response data for many cell lines [137]. Using cell line gene expression data all cell lines were evaluated and divided into two groups: those with high FBW7 expression, and those with low FBXW7 expression. The rational was that cell lines with low FBXW7 gene expression that are susceptible to a drug provide support that the given drug is a potential therapeutic for targeting the SL partners of FBXW7. In order to show this, the IC50 values for each group (high FBXW7 and low FBXW7) were used to generate a P-value (Mann-Whitney U test). The survival percentage data from the cancerRXgene database for each of 265 drugs at different concentrations was the basis for the dose-response curves. Out of 265 drugs, three were selected for further analysis based on the difference in susceptibility between the FBXW7 high and FBXW7 low cell lines: Cabozantinib (P-value = 0.033), Selumetinib (P-value = 0.006), and NSC-207895 (P-value = 0.099).

Chapter 5

Results

5.1 Construction of HYGIN predicted 10,419 potential SL interactions in humans for 1,009 human genes

Previously, a yeast genetic interaction network, The Cell Map, was described [23]. Using this information and a strict one-to-one ortholog mapping from yeast to human from InParanoid [109] (Supplementary Table B.1), we generated a refined network: HYGIN (Figure 5.1). HYGIN contains all of the yeast orthologs of human genes and their predicted SL interactions. Of the approximate 550,000 negative genetic interactions in yeast (Costanzo 2016; `http://thecellmap.org/costanzo2016/`, updated May 2016) we evaluated only statistically significant interactions with a P-value < 0.05 that were also strong negative interactions where $\epsilon < -0.2$. Using InParanoid mapping from yeast to human, the yeast network was reduced to statistically significant strong negative interactions that exist between the human orthologs of these yeast genes. The resultant HYGIN contains 1,009 genes and 10,419 proposed SL interactions (Supplementary Table B.2). Topology of this network combined with our Gene Ontology (GO) Slim terms (Supplementary Table B.3) shows dense clustering between genes involved in DNA damage and repair pathways and cell cycle regulators; or RNA processing and ribosome biogenesis and translation components, suggesting we have recovered meaningful humanized genetic interactions (Figure 5.1, Figure A.1). Since there are only 1,266 yeast genes that map to exactly one human ortholog, it is not surprising that the total number of human genes in the humanized network is less than that in the starting yeast network, as only a fraction of these genes have human orthologs.

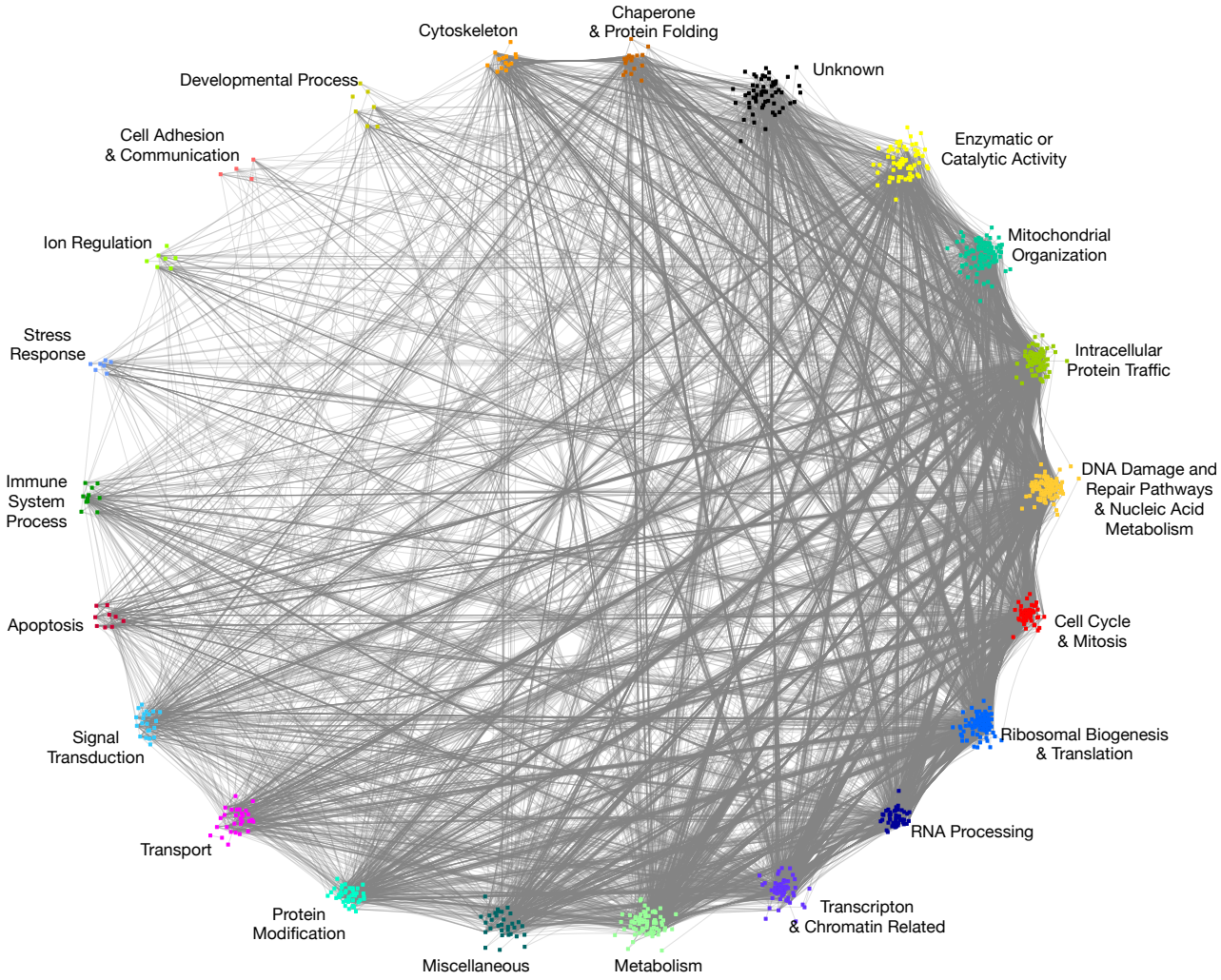

Figure 5.1: HYGIN network. Nodes are clustered around the perimeter of the network and coloured by GO slim terms which represent different biological processes. Each node represents one of the 1,009 human genes in the network. An edge between two genes represents a SL interaction translated from yeast. Denser areas of the network indicate a higher number of SL interactions between GO slim terms, while sparser areas of the network indicate fewer interactions between GO slims. Note that some areas of the network are sparser due to fewer genes representing a specified GO slim term and other areas of the network are over-represented increasing the number of edges in the network.

5.2 Validating the HYGIN network using SynLethDB

In order to validate the interactions in the HYGIN network *in silico*, the SL interactions in HYGIN were compared to the SynLethDB. The SL interactions in the SynLethDB are a combination of computationally predicted SL interactions, and SL interactions that have been identified through large or small-scale *in vitro* assays. Unfortunately, the text mining algorithm for SynLethDB did not accurately identify SL interactions from the literature. As a result they were all excluded from the validation of HYGIN. Of note, 6 SL entries in the SynLethDB identified through text mining were found to have some error or discrepancy (Table 5.1). Some of the errors were minor. For example, an SL interaction between *PSMD14* and *PSMD4* was in the SynLethDB despite the SL interaction being between *RPN11* and *RPN10* in the source manuscript. (RPN11 and RPN10 are synonyms for PSMD14 and PSMD4, respectively.) Other errors were major. A manuscript identifying yeast SL interactions was included in the SynLethDB and said to have human SL interactions; a manuscript that did not contain the words "synthetic", "lethal", or *POLD1* was included in the SynLethDB and recorded as having two SL interactions between *POLD1* and other genes; and most notably, a manuscript whose only reference to SL was in one of the references was identified by the SynLethDB to have 6 SL interactions. The data regarding these errors has been summarized and included as a table (Table 5.1), and the authors of the database were informed about the discrepancies and were eager to address them in the next version of SynLethDB.

Using the information in the database (except for text mining entries), we identified 11 SL interactions from HYGIN that have been identified by previous studies. Eight of these interactions have been computationally identified by DAISY [58], and 3 were identified by SynLethDB through analysis of data in DECIPHER [36]. The genes involved in these 11 interactions are summarized in Table 5.2. Since the number of SL interactions in common was low we wanted to test to see if these interactions could be the result of random chance or not. To do this we performed 100,000 iterations of the yeast-human ortholog mapping process starting with a random network that mimics the original yeast interaction network. It was found that there were 0 iterations that resulted in a humanized network having 11 or more SL interactions in common with the SynLethDB. However, there were random networks

PubMed ID	Description of error	SL interactions in SynLethDB
23651857	LSM2 does not appear in the manuscript and there is no direct mention of synthetic lethal interactions in the manuscript itself. The only reference to synthetic lethal interactions is one of the references: Luo J, Emanuele MJ, Li D, Creighton CJ, A1:G7 MR, Westbrook TF, Wong KK, Elledge SJ 2009. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 137: 835–848	*GSC LSM2* *LSM2 MYC* *LSM2 PHF5A* *GSC MYC* *GSC PHF5A* *MYC PHF5A*
16135799	FEN1 and FBXO18 do not appear in the manuscript. The manuscript is about yeast synthetic lethal interactions, not human synthetic lethal interaction.	*FBXO18 RAD51* *FBXO18 FEN1* *FEN1 RAD51*
25139395	POLD1 is not mentioned in the manuscript. Neither are the terms "Synthetic" nor "Lethal".	*POLD1 RAD51* *RAD51 RELA* *POLD1 RELA*
23980094	The only two valid synthetic lethal interactions in this manuscript are ASPSCR1 and PSMC2, and SMARCB1 and PSMA4. The other interactions for this manuscript listed in the database are just every possible combination of these two genes.	*ASPSCR1 PSMA4* *ASPSCR1 PSMC2* *PSMA4 PSMC2* *PSMA4 SMARCB1* *PSMC2 SMARCB1* *ASPSCR1 SMARCB1*
20941496	Valid SL interaction, although synonyms of the genes in the source manuscript are used: PSMD14 is a synonym for RPN11, and PSMD4 is a synonym for RPN10 where neither PSMD14 nor PSMD4 appear in the manuscript.	*PSMD14 PSMD4*
25334017	This manuscript is about embryonic lethality, not synthetic lethality. CDC45 only appears in the manuscript 3 times (once in the abstract and twice in the introduction), and is not described in the manuscript as involved in a synthetic lethal interaction.	*CDC45 GINS1* *CDC45 GINS3* *GINS1 GINS3* *GINS3 TAP2* *CDC45 GINS4* *GINS3 GINS4* *GINS4 TAP2* *CDC45 TAP2* *GINS1 TAP2* *GINS1 GINS4*

Table 5.1: List of SL interactions identified in the SynLethDB that were found to have errors. "PubMed ID" is the PubMed accession number of the source manuscript.

GeneA	GeneB	PubMedID	Source
KRI1	NOP56	25171417	DAISY
NCAPG	NCAPH	25171417	DAISY
TAF5	YEATS4	25171417	DAISY
DLD	SUCLA2	25171417	DAISY
CHAF1B	FEN1	25171417	DAISY
KIFC1	NCAPD2	25171417	DAISY
MCM6	WDHD1	25171417	DAISY
LIG1	RAD51	25171417	DAISY
GTF2H1	POLE	2667985	DECIPHER
PMS2	POLD1	2667985	DECIPHER
POLD1	POLE	2667985	DECIPHER

Table 5.2: List of SL interactions identified in HYGIN and the SynLethDB.

Number of Edges	Number of Networks
1	364,951
2	209,841
3	79,255
4	22,415
5	5,094
6	943
7	140
8	22
9	7
10	1

Table 5.3: A list of the number of edges in common between the SynLethDB and the 100,000 random networks used for validation. The number of edges column represents the number of edges in common between the two networks, and the number of networks column represents the fraction of random networks (out of 100,000) that contain that number of edges in common with the SynLethDB.

that had between 1 and 10 edges in common with the SynLethDB (Table 5.3). As a result, we can say with high confidence (P-value < 0.00001) that identifying these 11 interactions in common between HYGIN and the SynLethDB were not the result of random chance. Using this approach, we were able to show that the interactions in the network are statistically significant when compared to random networks.

5.3 Identification of down-regulated genes in breast cancer and building a breast cancer-specific SL interaction network

To make HYGIN more applicable for human cancer therapeutics, we focused on breast cancer and developed a breast cancer specific subnetwork. Exploiting any yeast SL interaction may not be beneficial if neither of the genes is altered in cancers. As a result, in order to identify those interactions in HYGIN that are relevant to breast cancer, we used TCGA data (`https://tcga-data.nci.nih.gov/tcga/`) to identify genes that are down-regulated in breast cancer. Although there are gene expression data for over 1,000 breast cancer patients available in TCGA, because gene expression is a relative measurement, we chose to use only those tumor samples that had matching normal gene expression data. That is, we used data only from patients where gene expression results were available for both cancer and normal tissue. This stringent approach was followed to eliminate those genes that are down-regulated in cancer, but whose expression in normal tissue is also low. In order to determine if genes were statistically down-regulated in breast cancer, we first had to determine which statistical test to use. The Shapiro-Wilk test for normality was used on the initial data and results showed that values from 80% of genes in the cancer dataset had normal distributions, compared to 56% of the genes in the dataset for normal tissue. These results indicated that despite being able to assume that the data followed a normal distribution [28], a non-parametric test was more appropriate for analyzing the data.

Our stringent procedure removed genes with any zero values for calculating gene up- and down-regulation in breast cancer. This was a very stringent method of analyzing the data,

and resulted in the removal of 6,593 genes from the initial dataset of 20,531 genes. The resulting 13,938 genes had non-zero values for both the cancerous and normal datasets for all 114 patients. Although this stringent cut-off potentially removed genes that are statistically down-regulated in breast cancer, it ensured a consistent sample size for all genes and reduced the potential for false positive results. Given the change in dataset, the Shapiro-Wilk test for normality was run again: 75% of expression values for cancer genes were normally distributed, compared to 46% of normal genes. Therefore, to be consistent across all data, the Wilcoxon signed-rank test was used. It determined statistical differences in the median gene expression values for each gene between the cancer and normal tissue.

For each patient, gene expression data for cancerous and normal tissue was compared to generate a log-2 ratio of cancerous to normal gene expression. By applying a 2-fold cut-off and using genes with statistically different medians (P-value after adjustment < 0.05), 1,745 genes were identified to be down-regulated in breast cancer (Figure 5.2, Supplementary Table B.4). Of these 1,745 genes that are statistically down-regulated in breast cancer, 181 were previously identified in the tumor suppressor gene database (TSG; `https://bioinfo.uth.edu/TSGene/`) as potential tumor suppressors.

The 1,745 genes that were statistically down-regulated in breast cancer were compared to the HYGIN network, and it was found that 15 genes were in both datasets. These 15 genes included some of the well-established tumor suppressors like *CAT* [35] and *FBXW7* [18]. The breast cancer specific subnetwork was generated using these 15 down-regulated genes and the 115 genes that they have SL interactions with for a total of 130 genes (Figure 5.3, Supplementary Table B.5). The 115 genes are themselves not down-regulated in breast cancer. By targeting the SL partners of the 15 genes that are already down-regulated in breast cancer, novel targeted therapeutics can be developed to provide treatment options for patients. A flow diagram of the methods used to analyze the breast cancer data and generate the breast cancer specific subnetwork is depicted in Figure 4.1. An interesting note is that these 15 genes were also found to be statistically down-regulated in breast cancer when all cancer samples were pooled and compared to all non-cancerous samples (Figure 5.4, Figure A.5, Figure A.6, Figure A.7).

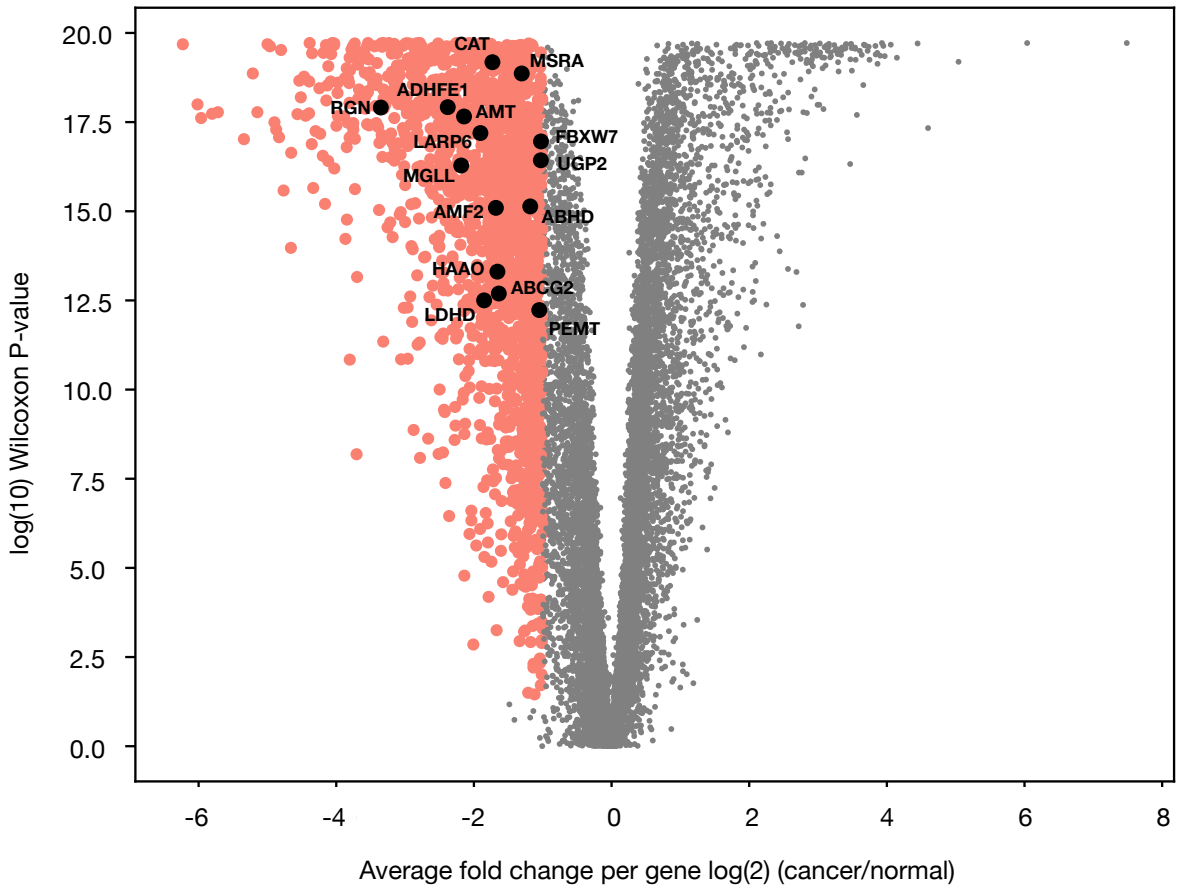

Figure 5.2: Volcano plot of the average change of expression per gene expressed as log(2)[cancer/normal] plotted against the log(10) of the adjusted Wilcoxon P-value. Dots in red represent genes that have an average 2-fold or more decrease in expression. Back dots represent the 15 genes that are also in the HYGIN network.

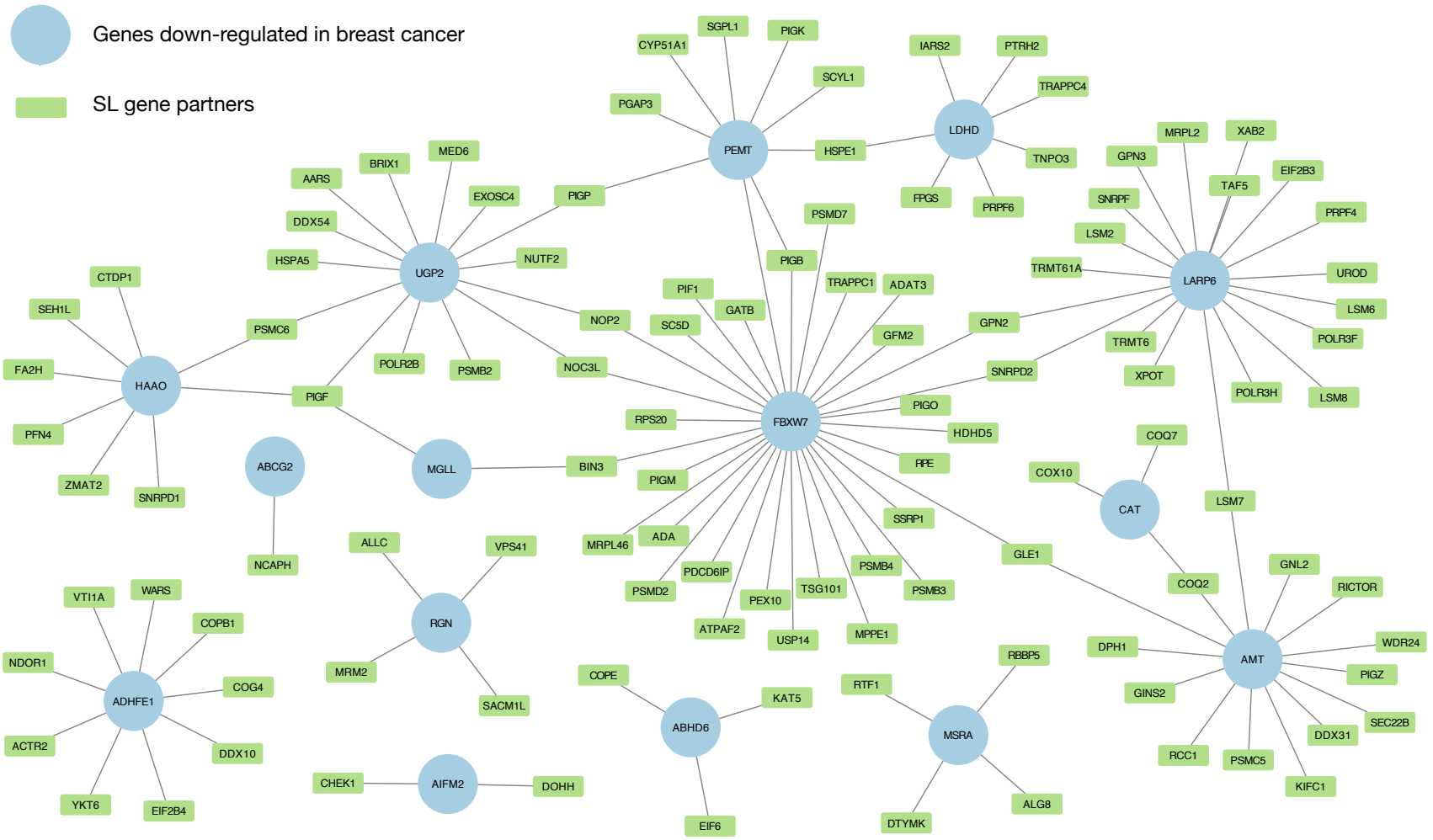

Figure 5.3: Visualization of the breast cancer specific subnetwork generated using 15 genes in HYGIN that were found to be down-regulated in breast cancer (blue circles) and their corresponding SL gene pairs. Edges represent proposed SL interactions between genes, and green nodes represent SL partners of the 15 genes.

5.4 Synthetic lethal interactions of *FBXW7* and their validation

Having identified *FBXW7* as a candidate gene worth exploring further, its yeast ortholog and expression in other cancers was investigated. The yeast gene *MET30* is an F-box protein containing five copies of the WD40 motif and is known to control cell cycle function as part of a E3 ubiquitin ligase complex [119]. According to InParanoid, FBXW7 is the human ortholog of MET30 that shares similar roles in humans [109] (Figure A.2) In fact, *FBXW7* is a well-known tumor suppressor that functions as a substrate-recognition protein within a SCF (SKP/CUL1/F-Box) E3 complex ubiquitin ligase complex, which targets numerous proteins for ubiquitin-mediated proteasomal degradation [130]. *FBXW7* is down-regulated not only in breast cancer, but also in 12 other cancers including colon, liver, lung, and prostate cancers (Figure 5.4, Figure A.3). However, *FBXW7* is also up-regulated in 6 other cancers including three types of kidney cancer, lung adenocarcinoma, and thyroid carcinoma (Figure 5.4, Figure A.3). Although SL interactions are potential targets for cancer therapeutics in some cancers, it would not be appropriate to use this strategy on all tumor types. Therefore, targeting SL interactions of *FBXW7* may have a wide opportunity for clinical application, but must be used in conjunction with genetic testing and patient gene expression data.

In order to validate the novelty associated with the identified SL interactions in breast cancer, we focused on SL interactions with *FBXW7*. Starting with *FBXW7* our analysis identified 32 SL interactions, many of which are proteasome components such as PSMB3, PSMB4, PSMD2, PSMD7, and USP14. Given that FBXW7 is a E3 ubiquitin ligase, these interactions highlight the genetic property of SL relationships where functional coherence is often observed. As cyclin E is a substrate of proteasome degradation, it is interesting to note that the recently published SL interaction between *FBXW7* and *CCNE1* may reflect the interaction between FBXW7 and proteasome components [4]. Our ortholog mapping is one-to-one and unfortunately, cyclin E falls into the"more than one ortholog" category in yeast and "more than one corresponding ortholog" in humans, and as a result was not included in HYGIN.

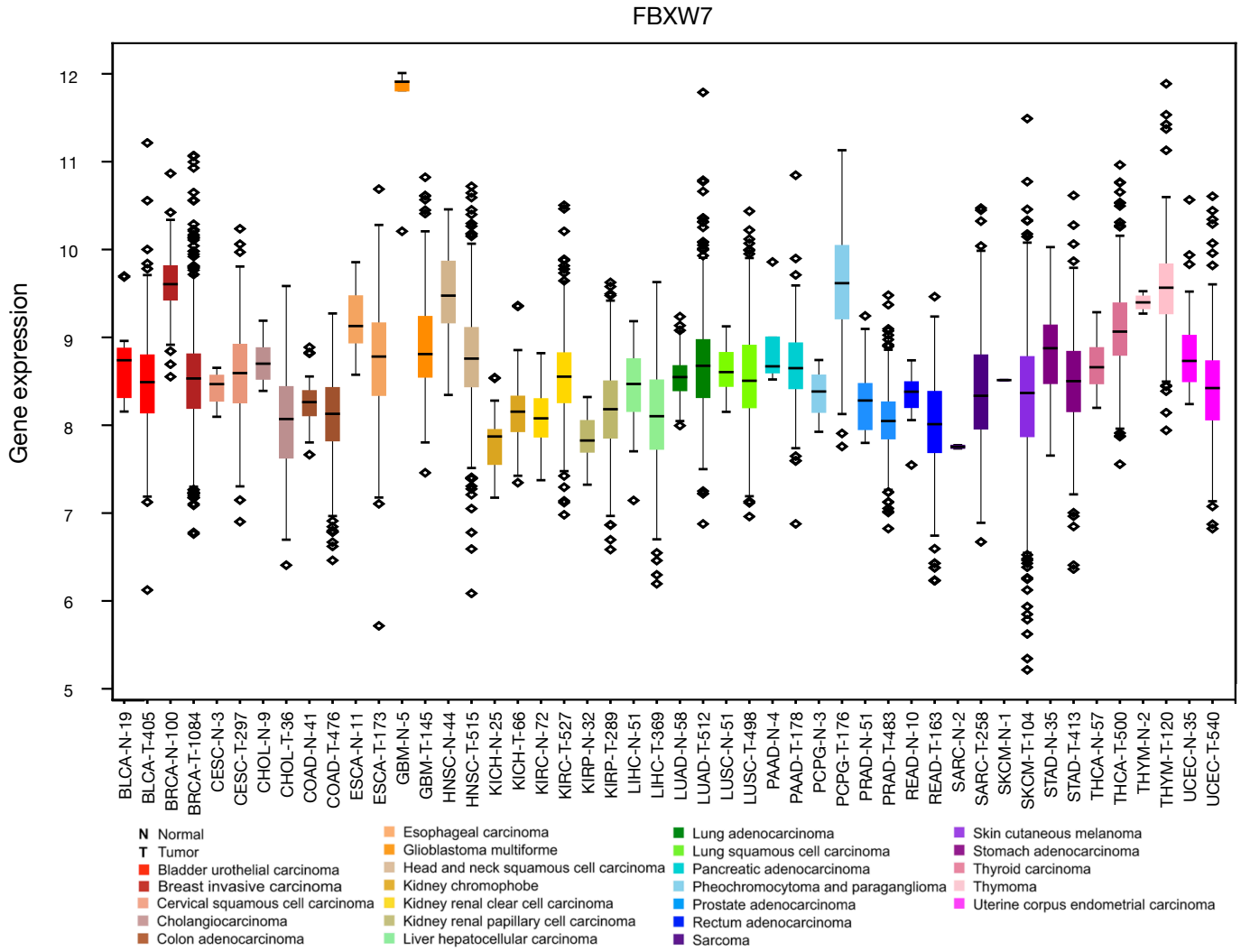

Figure 5.4: Gene expression of *FBXW7* in 24 cancer types and corresponding normal tissue as calculated from RNA-seq data by Expectation-Maximization (log2). The averages of normal and tumor expression of *FBXW7* for the same tissue type are plotted beside each other and colour-coded based on cancer type. Number of patient samples is indicated in the X-axis labels.

Additionally, *FBXW7* and *PEMT* are both separately down-regulated in breast cancer, yet they share a proposed SL interaction in HYGIN. The gene expression ratios for all 114 patients for both *FBXW7* and *PEMT* were evaluated to further investigate this finding. Results showed that 20% of patients have gene expression ratios greater than 2-fold for both *FBXW7* and *PEMT*, and one patient has gene expression less than 4-fold for both genes. This information suggested that the SL interaction between *FBXW7* and *PEMT* is a false positive result since the inhibition of both genes in a SL interaction should lead to cell death.

Our computational prediction suggested a SL interaction between *FBXW7* and *USP14*, a gene that codes for a deubiquitinating enzyme. FBXW7 has been proposed in the degradation of a number of substrates including Aurora B [20, 117]. Although Aurora B kinase is primarily degraded through the anaphase-promoting cyclosome complex (APC/c) [81], negative regulation of Aurora B by FBXW7 plays an important role in Aurora B ubiquitination and degradation [117]. USP14 is a major regulator of the proteasome and one of three proteasome-associated deubiquitinating enzymes, which also affects protein turnover in a substrate-specific manner [66]. Interestingly, a recent study has reported that over-expression of USP14 stabilized and prevented Aurora B degradation through deubiquitination [108]. Furthermore, a FBXW7-Aurora B-p53 negative feedback loop has been suggested [128]. This feedback loop suggests that a loss of *FBXW7* leads to an increase in Aurora B, which phosphorylates p53 and leads to MDM2 enhanced degradation of p53 and ultimately cancer cell growth [128]. While the loss of FBXW7 may already stabilize Aurora B [20, 117], over-expression of USP14 may be necessary for continued stability of Aurora B to maintain cell-cycle progression and cell survival. Thus, consistent with our observation, loss of USP14 when *FBXW7* is down-regulated may destabilize Aurora B, leading to a SL phenotype.

We also predict *FBXW7* to exhibit SL interaction with a member of the pentose phosphate pathway (PPP), ribulose-5-phosphate-3-epimerase (RPE), which catalyzes the reversible epimerization of D-ribulose 5-phosphate to D-xylulose 5-phosphate. Interestingly, previous work has shown that inhibition of the PPP results in decreased proliferation of tumor cell lines [12, 13, 19, 21, 98]. In addition, it has also been shown that the PPP is essential for metabolic network modulation to support tumor angiogenesis as inhibition of VEGFR-2 causes a decrease in PPP flux [126]. Thus, if inhibition of VEGFR-2 may decrease PPP flux, we hy-

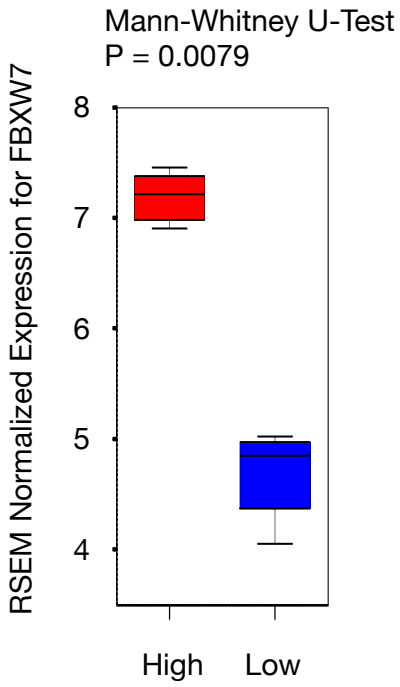

Figure 5.5: Gene expression data for *FBXW7* was extracted from all cell lines studied and cell lines were split into two categories: high *FBXW7* expression and low *FBXW7* expression. Here, a box plot shows *FBXW7* expression in all cell lines; up-regulated (red) and down-regulated (blue). The P-value (0.0079) represents the statistical difference between the two populations: cell lines with either high or low FBXW7 expression.

pothesized that inhibition of VEGFR should mimic the SL interaction between *FBXW7* and *RPE*. To test this idea, we used drug response data from the cancerRXgene database (`https://www.cancerrxgene.org`) and asked if cell lines deficient in *FBXW7* are sensitive to the VEGFR-2 inhibitor Cabozantinib. The cancerRXgene database contains data for 265 drugs and multiple cell lines and was examined to identify compounds that are more effective when used selectively with cell lines that have a low *FBXW7* expression. We found that cell lines with low expression of *FBXW7* were more sensitive to Cabozentanib (P-value = 0.033) (Figure 5.5, 5.6), which supports the idea that inhibition of VEGFR should mimic the SL interaction between *FBXW7* and *RPE*.

Similarly, we also found a SL interaction between *FBXW7* with *ADA*, an adenosine deaminase that regulates cellular levels of adenosine and deoxyadenosine. ADA, along with a few other enzymes (for example HGPRT), are responsible for purine metabolism and are

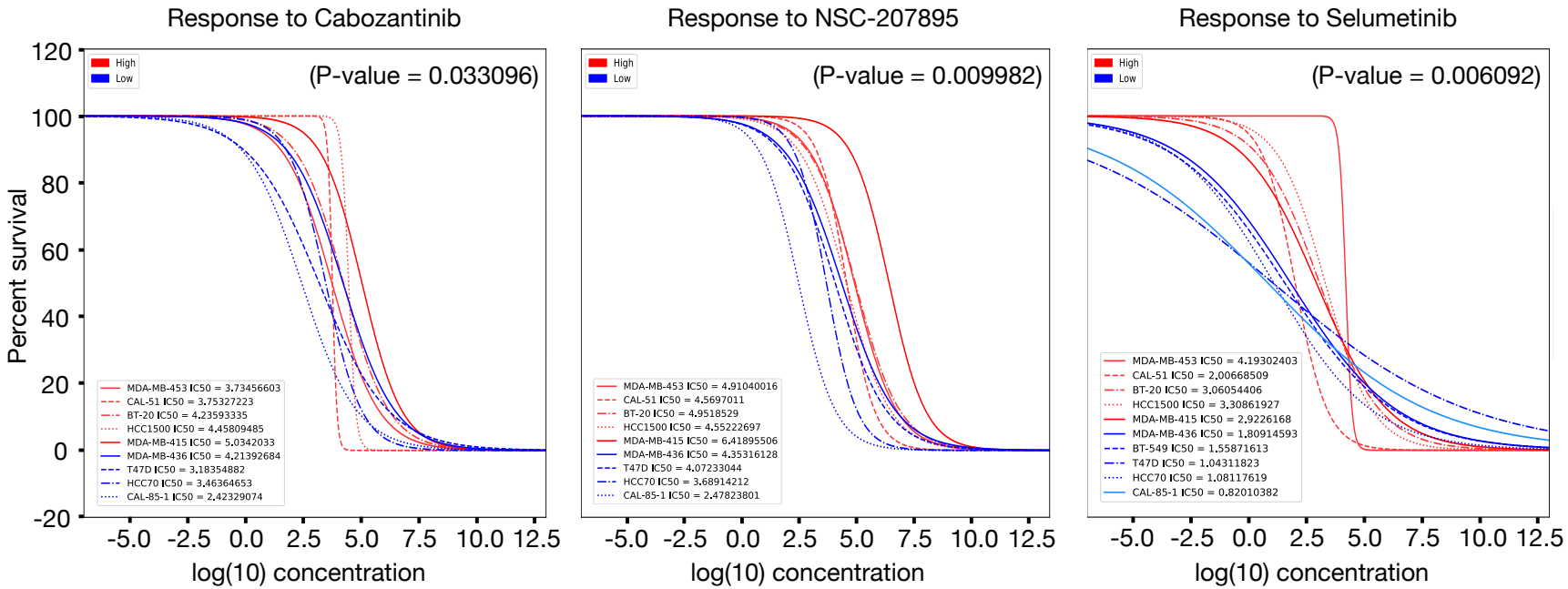

Figure 5.6: Cell line gene expression data and cell line drug data (cancerRXgene database) were evaluated to identify to which drugs low *FBXW7* cell lines are susceptible. Drug kill curves in blue represent cell lines where *FBXW7* is down-regulated, and drug kill curves in red represent cell lines that have up-regulated *FBXW7*. Drug curves for Cabozantinib (left), NSC-207895 (centre), and Selumetinib (right) were identified to have the greatest statistical significance between cell lines with high and low FBXW7 gene expression.

well known targets for cancer chemotherapy [38]. ADA can both degrade adenosine and bind extracellularly to adenosine receptors to function as an allosteric modulator to regulate adenosine. In fact, studies in a human astrocytoma cells have shown that manipulation of cellular purine metabolite concentrations can make these cells more sensitive to apoptosis [39]. While it remains to be explored if the astrocytoma cell line is deficient in *FBXW7*, these studies indicate a potential role for *ADA* as a therapeutic target. Interestingly, previous studies have shown that the expression of *ADA* is induced by growth factors like IGF in a Ras-MAPK pathway dependent manner [134]. To test this idea, we used drug response data from cancerRXgene database and asked if cell lines deficient in *FBXW7* are sensitive to the *MEK* inhibitor Selumitinib (Figure 5.6). Consistent with our SL observation, we found cells with low expression of *FBXW7* to be more sensitive to Selumitinib (P-value = 0.006). Thus, our observation strongly indicates that *FBXW7* can be used as a biomarker to indicate whether to treat patients with drugs like Selumitinib that indirectly affects the expression of *ADA*. Importantly, the availability of ADA inhibitors like 2'deoxycoformycin (Pentostatin) that are already in the clinics as standard chemotherapeutic agents for lymphoid malignancies[83] may also represent a potential option to test our prediction. Indeed, analyses of the drug data also indicates a potential opportunity for the MDM2 inhibitor NSC-207895 in treating FBXW7 deficient cancers (P-value = 0.099, Figure 5.6). Using an MDM2 inhibitor to treat *FBXW7* deficient cancers is interesting because MDM2 is an negative regulator of p53 [64]. Previous research has also showed that *FBXW7* is a transcription target of p53, and that *FBXW7* is dramatically up-regulated when p53 is introduced into p53-defficient cells by adenovirus [62]. Therefore, if we use an MDM2 inhibitor, we would expect an increase in p53 and subsequently an increase in *FBXW7*, which would mean that targeting *FBXW7* SL interactions would be not be effective in this instance. However, there is also evidence that supports FBXW7 mediating the role of P53 in response to DNA damage [72]. As a result, although the *in silico* drug analysis suggests that an MDM2 inhibitor would be a valuable drug for treating *FBXW7* deficient breast cancers, additional *in vitro* analyses are imperative in order to understand what is going on inside the cell.

Chapter 6

Discussion

In this thesis we describe a novel approach to identifying potential SL interactions in breast cancer that uses a yeast SL interaction network, yeast-human gene ortholog information, and patient gene expression data. Yeast is a common model organism and generating a human orthologous network from a yeast gene network provides a major framework to extend yeast genetic interaction data to humans. When generating HYGIN, strict one-to-one yeast-human ortholog mapping was used to avoid ambiguity as there are several instances where a single yeast protein maps to multiple human orthologs, and vice versa. HYGIN is cancer independent as no assumptions about cancer are made while generating the network. As a result, any cancer gene expression data can be subsequently applied to generate cancer type-specific subnetworks of genetic interactions. In this thesis we generate a breast cancer specific subnetwork of HYGIN using breast cancer patient data from the TCGA.

6.1 Network generation

The development of HYGIN was based on previous work by Deshpande *et al.*, but took into consideration some of the problems with their network to generat a more robust and strict network of human SL interactions [27]. Previously, Deshpande *et al.* generated two networks: a complete SL network using all available orthologs in InParanoid, and a cancer-specific network. The first network did not discriminate between any of the yeast-human orthologs in InParanoid and simply applied any available orthologs (whether they were one-to-one, one-to-many, many-to-one or many-to-many) to the yeast data. The second network, used strict one-to-one yeast-human ortholog mapping network and contained cancer-relevant mutation data. This thesis identified several discrepancies in their initial larger network

related to problems with Ensembl identifiers, accession numbers, and gene names of the SL interactions. Initially it was thought that these errors were the result of inconsistent bookkeeping. However, perspective and more information suggested that these mistakes are due to changes in the databases (over the past 8 years) as well as updates to the human genome, including characterization of previously uncharacterized genes and proteins. This brought to light an interesting problem for HYGIN: how to make this research stand the test of time to the best of our ability now? Researchers do not anticipate that entries in a database or a reference table change over time or become deprecated; however, it was something to consider when developing HYGIN: how to ensure that the network would be relevant in the future? As a result, HYGIN has two reliable identifiers for each entry. In addition to the gene name, a UniProtKB identifier is also present for each protein made from that gene. Since UniProtKB is a fundamental bioinformatics database heavily utilized in current research, any deprecated identifiers will be maintained in the database, with pointers to updated identifiers. At the very least, deprecated identifiers will be recorded in the entry for a particular protein, and that protein can still be located using the deprecated identifier as a search term.

The Deshpande *et al.* network also has 48 instances of non-canonical names used in the common name list that contain either '-' or '.' and a version number as part of the name. For example, the network contained the name "06-Mar". It is known that this name can be the result of automatic formatting when "MAR6" is typed into Excel [140]. It is still possible that there are outliers in gene and protein accession lists that were used in this thesis, and that there are minor mistakes in HYGIN. However, steps were taken to ensure that these anomalies were minimized.

It is also worth discussing the differences in ortholog mapping between HYGIN and the Deshpande *et al.* research. For example, in the instance where there are multiple human orthologs for a single yeast protein, a corresponding node in the Deshpande *et al.* network now represents multiple human proteins. If we are attempting to compare their network to previously identified SL interactions, we are left with a challenge. All of the nodes can be "unpacked" such that there is now a node representing each protein and a new edge from each of these proteins to its SL partners. However, this "unpacking" of complex nodes can drastically increase the number of edges and nodes in the network. In the case of the Desh-

pande *et al.* network, the number of edges would quickly double from the addition of these new edges resulting in a new network that has more than 50,000 edges. This would generate a large number of potential falsely identified SL interactions in humans as well. However, the second network generated by Deshpande *et al.* was a cancer-specific one that only used one-to-one ortholog mapping for higher stringency. The potential problems identified in the Deshpande *et al.* work was one of the motivations to use strict one-to-one ortholog mapping for HYGIN.

Furthermore, HYGIN used the same cut-offs ($P < 0.05$ and $\epsilon < -0.2$) as the Deshpande *et al.* network. However, they also used a relaxed cut-off ($P < 0.05$ and $\epsilon < -0.08$) for SL interactions that were identified in reciprocal screens (where query A was crossed with array B and vice veresa [22]). In our work to maintain stringency we maintained the same cut-off ($P < 0.05$ and $\epsilon < -0.2$), irrespective of whether an interaction is identified in a reciprocal screen or not. Their work was compared to HYGIN to determine if there were more interactions identified in our work. Compared to Deshpande *et al.* [27] who identified 1,522 potential SL interactions in humans, we identified 10,419 interactions. Interestingly, there are no SL interactions in common between these two networks. A potential reason that HYGIN contains more SL interactions is that our study uses a more recent version of The Cell Map database that includes interactions between essential and nonessential yeast genes. Another reason is that their aim was to generate a SL gene network involving cancer-relevant mutations in human cells. In contrast, our aim was to generate a SL gene network in a cancer independent context, then apply cancer gene expression data to identify SL interactions in breast cancer. As a result, the SL interactions in their network have cancer relevant mutations, whereas no assumptions about cancer were made in the generation of HYGIN.

6.2 Statistical analysis

There was trial and error involved in analyzing the TCGA data for the breast cancer sub-network. Similar to the initial HYGIN, the initial analysis of the breast cancer data lacked statistical validity. As a result, in order to have more confidence in our results, we applied

strong statistical methods to the data which yielded a more concise breast cancer subnetwork. The final methods for data analysis involved removing genes with any zero expression values, evaluating an expression ratio for each gene for each patient, statistically determining if the expression was down-regulated (Wilcoxon signed-rank test), multiple hypotheses adjustment (Benjamini-Hochberg procedure), and setting a cut-off value for genes identified to be down-regulated in breast cancer (biological cut-off of 2-fold loss of expression).

An interesting result was observed while evaluating the breast cancer gene expression data to determine genes that were down-regulated in breast cancer. The expression of the 15 genes that were identified to be in HYGIN and down-regulated in breast cancer were also analyzed in 24 different cancer types. This analysis used pooled normal versus pooled cancerous tissue in order to determine if a gene was statistically up- or down-regulated in the other cancer types. Though previously it was discussed that a method using pooled data is less stringent (Section 6.1), it is worth noting that in breast cancer, even when all 1,084 cancer samples were pooled and compared to all 100 normal samples, all 15 genes were still statistically down-regulated. This could largely be due to sample size, and caution should be taken when using this method for other cancer types that have fewer normal tissue gene expression datasets. Using this data as a starting point to explore these 15 genes and their SL interactions as methods of treatment in additional cancer types is promising.

6.3 Network validation

When validating HYGIN we used previously identified SL interactions in humans and took an *in silico* approach to validation rather than an *in vitro* approach. Though *in vitro* work is still required to successfully validate that the interactions in HYGIN are biologically relevant, the *in silico* validation provides support for our results. Of the nearly 35,000 SL interactions recorded in the SynLethDB, 5,830 were identified with DAISY, an *in silico* approach to the identification of SL and synthetic dosage lethal (SDL) interactions in humans [58]. DAISY uses both patient and cell line data to computationally predict SL and SDL interactions using three inference strategies. Yet only 8 of these SL interactions identified by DAISY were found in HYGIN. One explanation for this is that HYGIN is cancer independent. As a result,

relying solely on yeast orthologs in humans and previously identified SL interactions in yeast, human genes without yeast orthologs were excluded from our network. In contrast, DAISY evaluated existing cancer gene expression data, cancer mutation data, and cancer cell line data to computationally predict SL interactions. When comparing the number of genes in each of the studies, HYGIN analyzes 1,009 genes and DAISY more than 20,000 genes. This difference in sample size alone could account for the lack of overlap between the two studies. However, it is interesting that DAISY did not predict more interactions between human genes with known yeast orthologs. One reason for this could be the focus on cancer gene expression, mutation, and cell line data that drive DAISY towards potential cancer-relevant SL interactions and away from SL interactions involving non-cancerous genes.

There were also 1,020 SL interactions as a result of analyzing the shRNA data in the DECIPHER database [45]. Since the DECIPHER database does not contain the genetic information of healthy individuals, SL interactions predicted from the analysis of bi-specific shRNA screens also have a tendency to identify SL interactions involving known cancer oncogenes and tumor suppressors. Therefore, it is not surprising that there were only 3 SL interactions identified in both HYGIN and DECIPHER given the cancer independent nature of HYGIN, and the small number of patient-specific SL interactions identified in DECIPHER.

The 11 interactions from two separate sources provide evidence that there are SL interactions in HYGIN that have been previously identified in humans. Although it would be arduous to exhaustively test all 10,419 SL interactions in the network *in vitro*, these 11 suggest that HYGIN has identified a potential 10,408 SL interactions between 1,009 genes that have not previously been reported in the SynLethDB.

6.4 Breast cancer subnetwork

The breast cancer specific subnetwork contains several SL interactions involving genes that were determined to be down-regulated in breast cancer patients. The subnetwork, and ultimately this subset of SL interactions, are an ideal starting point for *in vitro* and *in vivo* validation studies and suggest novel targeted therapeutic strategies. Although there are only 130 interactions in the breast cancer subnetwork, validating all these interactions would be

a time- and resource-consuming task. In contrast, additional *in silico* analyses is a more cost- and resource-effective approach to identify SL candidates to test *in vitro*. By extracting 15 genes that were found to be down-regulated in humans and their SL partners, we can target their SL partners with known or novel drugs to identify new treatment strategies on a patient-by-patient basis.

The breast cancer specific interaction network produced 130 SL interactions between 15 genes down-regulated in breast cancer and their 115 SL partners. We further analyzed the 32 genes that have proposed interactions with the tumor suppressor *FBXW7* and found that five of those genes have previously identified drug inhibitors. The remaining genes that interact with the 15 down-regulated genes in the breast cancer network are also potential targets for further studies.

FBXW7 and *PEMT* are both down-regulated in breast cancer and they share a proposed SL interaction in HYGIN. The gene expression ratios for all 114 patients for both *FBXW7* and *PEMT* were evaluated to further investigate this finding. Results showed that 20% of patients have gene expression ratios less than 2-fold for both *FBXW7* and *PEMT*, and one patient had gene expression less than 4-fold for both genes. There are two possibilities that could explain this result. First, it is possible that at some point in evolutionary time this SL interaction was valid. However, the cancer cells overcame the SL dependency of these two genes in humans, and what we expect to manifest as cell death results in cell survival. Second, it is possible that this result is a false positive. In light of a study that showed that there was only 23% overlap in SL interactions between two species of the same genera (*Saccharomyces cereviseae* and *Saccharomyces pombe*) [29], SL relationships are lost over evolutionary time. Thus, we are expecting to see a loss of some SL interactions in our network as a result of a larger species gap between *Homo sapiens* and *Saccharomyces cerevisieae*. Ultimately, this result highlights the need for biological testing of predicted SL interactions prior to beginning therapeutic trials. Though the HYGIN network is based on previous valid research methods, human tissue behaves differently than predicted results.

The breast cancer specific network contains interactions that are valuable starting points for SL research moving forward and provides numerous SL interactions for the scientific community to study. The 15 genes that were identified to be down-regulated in breast cancer

were also assessed in 24 other cancer types. Of the 15 genes, 11 of them were statistically down-regulated in more than 15 cancer types including breast cancer (Figure A.4, A.5, A.6, and A.7). Of note, *HAAO*, *MGLL*, and *UPGE* were down-regulated in 18 cancer types; *ADHFE1*, *CAT*, and *MSRA* were down-regulated in 19; and *RGN* was down-regulated in 21 different types of cancer. This information suggests that SL interactions identified in breast cancer for these genes would be extremely valuable to explore in other cancers as well. We predict that *in vitro* and *in vivo* studies for these genes and their SL partners in cancer models could lead to novel treatments for breast cancer that could be extended to other cancer types.

6.5 Limitations of this study

Although in this thesis the HYGIN network was generated and validated, it is important to address some of the limitations of this approach and how they impact this work moving forward. First and foremost, it is important to acknowledge that the initial yeast dataset represents a small portion of the potential interactions in the human genome. This was made evident through the ortholog mapping, since only 1,009 yeast genes have strict one-to-one human orthologs. As a result, we are essentially comparing approximately 16 - 20% of the yeast genome to 5% of the human genome. This reduction in sample space is extremely advantageous for saving time in the laboratory, but can result in overlooking numerous potential SL interactions. Additionally, using strict one-to-one ortholog mapping also affects the number of genes present in HYGIN. There are more than 1,000 additional yeast genes that have one-to-many or many-to-one orthologous relationships with human genes. The TCGA analysis eliminated any genes that had a non-zero value for any patient (either cancer or normal datasets) which resulted in approximately 30% of human genes being excluded in the breast cancer down-regulation analysis. Furthermore, using stringent P-value cut-offs and a 2-fold cut-off for down-regulation also potentially excluded a number of potential genes and their SL interactions from the analysis. Therefore, although this analysis identified potential candidates for patient specific therapeutics, it very likely missed many other potential relevant SL interactions, and there is room for expansion and improvement moving forward.

Chapter 7

Future Work and Conclusions

7.1 Future Work

Throughout the work for this thesis a large amount of data was analyzed and used to generate HYGIN and the breast cancer specific subnetwork. However, only a small portion of the results were analyzed in great detail: *FBXW7* and its SL partners. As a result, the remaining 14 genes down-regulated in breast cancer and their 105 SL partners, the remaining 1,730 genes down-regulated in breast cancer, and the remaining 10,289 SL interactions in HYGIN were not analyzed in detail. This section discusses the potential future analyses of the data generated through this project, potential caveats of future analyses and benefits to patient care as a result.

7.1.1 Analyzing the 15 genes down-regulated in the breast cancer subnetwork

In addition to evaluating the role of *FBXW7* and its SL partners in breast cancer, future work could also apply the same analyses to the remaining 14 genes that are down-regulated in the breast cancer subnetwork. The range of SL partners for these 14 genes is 1 to 19, with 7 genes having fewer than 5 SL partners (Figure A.3). Analyzing the 7 genes that have more than 5 potential SL partners that can be used as drug targets (*LDHD*, *HAAO*, *ADHFE1*, *PEMT*, *AMT*, *UGP2*, *LARP6*) could lead to additional treatment strategies for breast cancer patients. Moreover, patients who have more than one of these genes down-regulated in their tumor could benefit from combined drug therapy, which could increase the effectiveness of treatment and recovery. The same method that was used to identify *FBXW7*

deficient cell lines that were susceptible to known chemotherapeutics can also be applied to these 14 genes. This analysis method would provide a similar amount of data for each of these genes, which could lead to repurposing current therapeutics on a patient-by-patient basis. This data could also provide insight into the mechanism behind some of the SL interactions that were identified. Ultimately, however, this information could provide insight into which genes are the best candidates for *in vitro* and *in vivo* studies moving forward by highlighting genes that have the most clinical relevance and potential for success. Given that there are 130 SL interactions in the subnetwork, narrowing in on one or two genes to study *in vitro* or *in vivo* would be resource- and time-efficient, especially if there was sufficient evidence to support drug susceptibility in cell lines deficient in these genes.

7.1.2 Expand methods to other cancer types

Since the TCGA database has the same format for all of the cancer types and the Vizea-coumar research group routinely downloads the most up-to-date version of all of the TCGA data, running the same analysis on the remaining 23 cancer types in the TCGA is very straightforward. However, the biggest challenge arises from the lack of normal data for some of the cancer types. For this study, we were fortunate enough to be able to use the dataset from the TCGA database with the most paired samples (114). Other cancer types in the TCGA database have between 1 and 72 paired cancerous and normal samples. In instances where there are 72 normal samples the argument could be made that sample size is large enough for statistical validity. However, in instances where there are only one or two normal samples, it is difficult to analyze the data with statistical confidence. As a result, analyzing the remaining cancer datasets can be prioritized based on the number of paired samples available. The breast cancer dataset had the largest number of paired samples, and the next top 5 datasets are: kidney renal clear cell carcinoma (KIRK-US): 72 samples, thyroid carcinoma (THCA-US): 58 samples, lung adenocarcinoma (LUAD-US): 55 samples, prostate adenocarcinoma (PRAD-US): 52 samples, and liver hepatocellular carcinoma (LIHC-US): 48 samples. This data combined with the gene down-regulation data from breast cancer could be very useful to evaluate how different cancer types (based on tissue) are similar (based on genetic profiles). If we are able to classify tumors based on genetic profiles and exploit

current therapies that are based on cancer type, there is the potential to repurpose several clinically relevant chemotherapeutics for patient specific therapeutics.

7.1.3 The 1,745 genes down-regulated in breast cancer

Analysis of the TCGA data generated a list of 1,745 genes that were down-regulated in breast cancer, of which only 15 were identified in HYGIN and used as the basis for the breast cancer subnetwork. The remaining 1,730 genes that were identified have not been analyzed. Potential further analyses include comparing these genes to the SynLethDB to see if any of these genes have been identified to have SL partners in other studies or if other cancer types are analyzed, comparing the set of down-regulated genes across cancer types in order to identify common genes for novel drug development. They could also be used as a starting point for SL analysis *in vitro*. Genes of interest could be screened against the entire human genome in order to identify the complete set of SL interactions for any given gene that is down-regulated in breast cancer. The results would provide valid drug targets that can be exploited for patient therapeutics.

7.1.4 Additional FBXW7 analyses

The analyses surrounding *FBXW7* in this thesis are mostly computational. Although the drug data in the cancerRXgene database is from previous cell line experimentation, no wet lab experimentation was completed directly for the purpose of this thesis. There has been previous research that has identified SL interactions with *FBXW7 in vitro* [106], although there was no overlap between the 32 SL interactions identified in this thesis and that study. As a result, the natural progression for this research is to test *FBXW7* and its SL interactions *in vitro* and *in vivo* to provide biological evidence for these interactions.

7.2 Conclusion

Yeast genetic interaction data and orthology mapping was used to generate HYGIN, a humanized SL interaction network, containing 10,419 proposed SL interactions between 1,009

human genes. HYGIN improves on previous attempts to identify human SL interactions using yeast data by using more stringent and rigorous analyses. Cancerous and normal tissue data from the TCGA were evaluated to identify genes that were down-regulated in breast cancer. The genes that were in HYGIN and down-regulated in breast cancer were used to generate a breast cancer specific subnetwork containing 130 genes and 130 proposed SL interactions. The tumor suppressor FBXW7 was identified to have the most SL interactions in the breast cancer subnetwork and was selected for further study. *In silico* cell line and drug analysis was used to identify potential drugs for therapeutics when FBXW7 is down-regulated. Given that our hypothesis was to identify gene targets for breast cancer therapeutics, identifying 32 potential novel drug targets for FBXW7 deficient breast cancers makes this project a success. These findings provide the basis for further testing of SL interactions with FBXW7 *in vitro* and *in vivo* to validate them for potential patient therapeutics.

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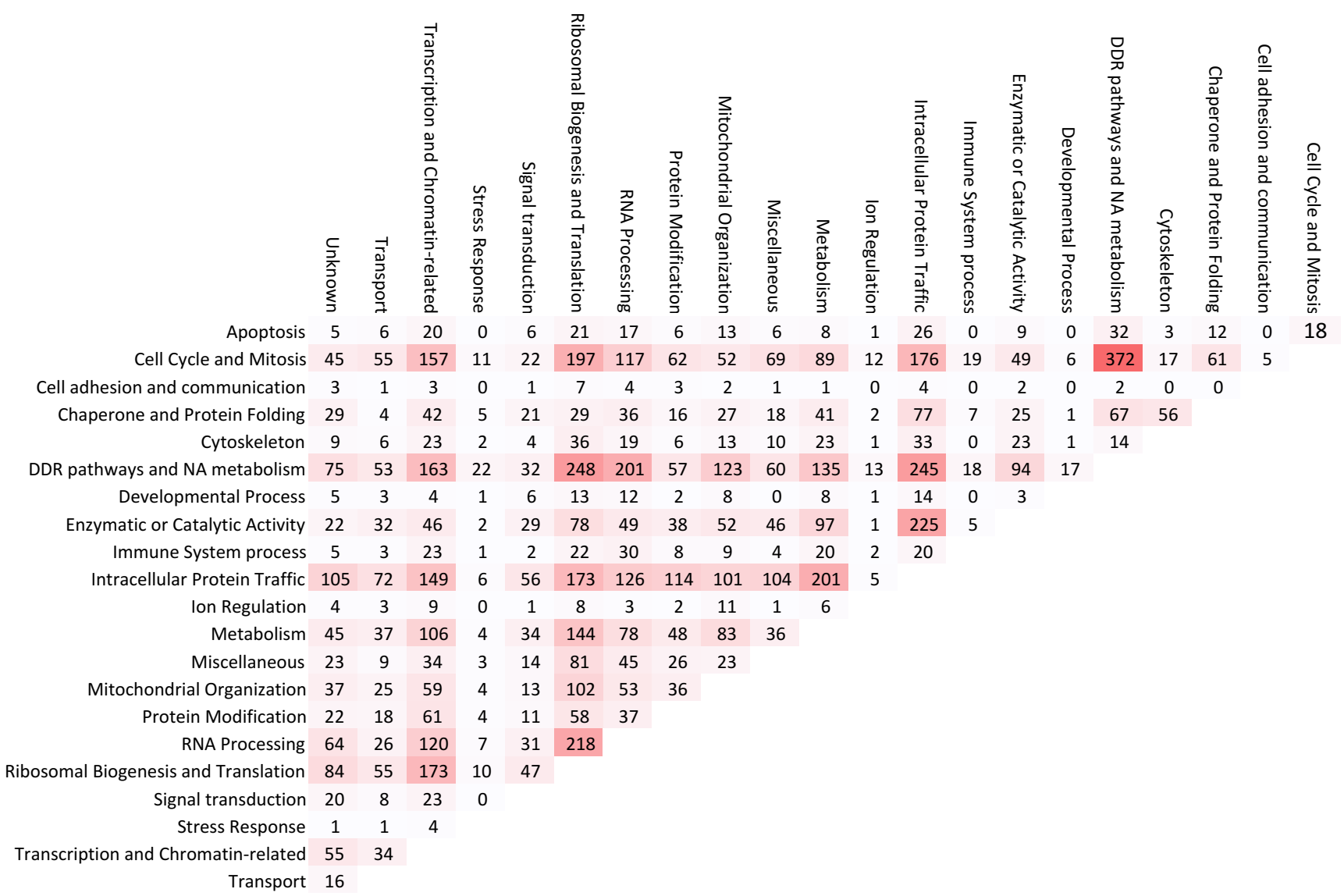
Appendix A

Supplemental Figures



	Unknown	Transport	Transcription and Chromatin-related	Stress Response	Signal transduction	Ribosomal Biogenesis and Translation	RNA Processing	Protein Modification	Mitochondrial Organization	Miscellaneous	Metabolism	Ion Regulation	Intracellular Protein Traffic	Immune System process	Enzymatic or Catalytic Activity	Developmental Process	DDR pathways and NA metabolism	Cytoskeleton	Chaperone and Protein Folding	Cell adhesion and communication	Cell Cycle and Mitosis
Apoptosis	5	6	20	0	6	21	17	6	13	6	8	1	26	0	9	0	32	3	12	0	18
Cell Cycle and Mitosis	45	55	157	11	22	197	117	62	52	69	89	12	176	19	49	6	372	17	61	5	
Cell adhesion and communication	3	1	3	0	1	7	4	3	2	1	1	0	4	0	2	0	2	0	0		
Chaperone and Protein Folding	29	4	42	5	21	29	36	16	27	18	41	2	77	7	25	1	67	56			
Cytoskeleton	9	6	23	2	4	36	19	6	13	10	23	1	33	0	23	1	14				
DDR pathways and NA metabolism	75	53	163	22	32	248	201	57	123	60	135	13	245	18	94	17					
Developmental Process	5	3	4	1	6	13	12	2	8	0	8	1	14	0	3						
Enzymatic or Catalytic Activity	22	32	46	2	29	78	49	38	52	46	97	1	225	5							
Immune System process	5	3	23	1	2	22	30	8	9	4	20	2	20								
Intracellular Protein Traffic	105	72	149	6	56	173	126	114	101	104	201	5									
Ion Regulation	4	3	9	0	1	8	3	2	11	1	6										
Metabolism	45	37	106	4	34	144	78	48	83	36											
Miscellaneous	23	9	34	3	14	81	45	26	23												
Mitochondrial Organization	37	25	59	4	13	102	53	36													
Protein Modification	22	18	61	4	11	58	37														
RNA Processing	64	26	120	7	31	218															
Ribosomal Biogenesis and Translation	84	55	173	10	47																
Signal transduction	20	8	23	0																	
Stress Response	1	1	4																		
Transcription and Chromatin-related	55	34																			
Transport	16																				

Figure A.1: 22 GO Slim terms and the interactions between them as translated from yeast are represented in this table. Cells are colour-coded according to the number of interactions between two groups of GO terms, where darker cells represent more interactions and lighter cells represent fewer interactions.

A

Searching the species
Homo sapiens
for the identifier **met30** excluding inparalogs scoring below 0.05

xml

Inparalog and Orthologs cluster for Saccharomyces cerevisiae and Homo sapiens

Cluster 817

Protein ID	Species	Score	Bootstrap	Description	Alternative ID
P39014	Saccharomyces cerevisiae	1	43%	F-box protein MET30	MET30_YEAST (UniProt)
Q969H0	Homo sapiens	1	99%	F-box/WD repeat-containing protein 7	FBXW7_HUMAN (UniProt)

B

Searching the species
Homo sapiens
for the identifier **cdc4** excluding inparalogs scoring below 0.05
More than one protein found:

Inparanoid	Gene ID	Protein ID	Species	Description	Alternative ID
Cluster	CDC4	A3LV96	Scheffersomyces stipitis	F box protein, for ubiquitin-dependent degradation	A3LV96_PICST (UniProt)
Cluster	CDC4	Q5A9A6	Candida albicans	Potential ubiquitin ligase F-box subunit	Q5A9A6_CANAL (UniProt)

Figure A.2: Two searches of the InParanoid database were conducted to verify that FBXW7 has a strict one-to-one mapping. A) The human genome was searched for the protein MET30 (the yeast protein that is orthologous to FBXW7 in humans). As expected, a single ortholog mapping was identified between *Saccharomyces cerevisiae* and *Homo sapiens*. B) The protein CDC4, a synonym for FBXW7, in humans was subsequently searched for in humans. Finding no ortholog in human, InParanoid reported "hits" in other organisms. Results showed that there is no entry for CDC4 in humans, which means that there is no additional FBXW7 ortholog in *Saccharomyces cerevisiae*.

Gene Name	Bladder urothelial carcinoma	Breast invasive carcinoma	Cholangiocarcinoma	Colon adenocarcinoma	Esophageal carcinoma	Glioblastoma multiforme	Head and neck squamous cell carcinoma	Kidney chromophobe	Kidney renal clear cell carcinoma	Kidney renal papillary cell carcinoma	Liver hepatocellular carcinoma	Lung adenocarcinoma	Lung squamous cell carcinoma	Pheochromocytoma and paraganglioma	Prostate adenocarcinoma	Rectum adenocarcinoma	Stomach adenocarcinoma	Thyroid carcinoma	Uterine corpus endometrial carcinoma
ABCG2	5.88E-05	2.48E-46	2.15E-06	2.06E-25	NS	NS	5.88E-04	4.82E-02	9.48E-07	7.70E-15	5.47E-12	4.36E-31	4.88E-24	2.22E-02	1.14E-11	2.28E-07	1.48E-03	7.29E-04	9.46E-20
ABHD6	3.12E-04	3.84E-39	3.22E-06	4.48E-22	6.29E-04	1.02E-03	NS	6.60E-06	NS	NS	6.36E-21	7.45E-30	6.47E-22	1.07E-02	2.57E-18	1.55E-05	1.24E-10	7.18E-11	2.16E-09
ADHFE1	8.84E-08	1.06E-54	2.15E-06	3.44E-25	1.15E-06	9.57E-04	3.12E-09	4.57E-06	4.22E-26	NS	5.55E-14	9.48E-14	2.31E-17	5.58E-03	NS	2.93E-07	3.84E-16	2.33E-14	6.87E-17
AIFM2	NS	1.47E-30	NS	9.61E-03	NS	NS	NS	3.22E-10	7.76E-05	2.87E-02	1.03E-12	6.83E-13	NS	1.10E-02	2.68E-15	NS	6.47E-03	1.00E-12	4.49E-12
AMT	5.01E-04	1.90E-34	2.15E-06	6.14E-15	1.74E-05	NS	NS	2.21E-07	6.21E-29	4.58E-08	4.05E-12	NS	4.26E-11	NS	4.87E-12	1.67E-02	3.73E-10	4.75E-02	2.00E-03
CAT	2.20E-05	1.16E-60	2.15E-06	8.34E-19	1.71E-04	1.17E-02	3.51E-07	NS	8.33E-34	1.28E-16	9.87E-25	1.29E-35	5.58E-32	1.66E-03	2.19E-09	6.83E-05	1.13E-08	2.62E-02	4.11E-07
FBXW7	2.37E-02	3.48E-57	4.50E-04	2.17E-02	4.87E-03	8.77E-05	1.56E-12	4.58E-05	4.88E-20	7.95E-06	1.09E-05	1.60E-02	4.53E-02	2.59E-03	3.69E-06	1.38E-02	3.09E-04	1.08E-12	8.50E-07
HAAO	6.77E-12	3.16E-40	2.15E-06	1.74E-03	NS	5.75E-03	1.08E-08	6.29E-10	9.83E-06	9.35E-03	3.65E-19	1.90E-09	1.50E-09	3.38E-02	1.21E-14	NS	1.94E-03	6.98E-04	2.63E-17
LARP6	8.70E-05	2.46E-53	3.56E-05	NS	1.88E-03	9.77E-05	6.33E-04	3.42E-06	3.86E-11	1.74E-02	3.96E-03	1.13E-07	3.13E-10	2.68E-03	8.67E-04	NS	2.78E-07	NS	5.28E-11
LDHD	NS	7.42E-31	2.15E-06	1.37E-25	4.23E-05	9.77E-05	3.86E-19	1.09E-07	6.10E-31	9.57E-04	6.55E-23	1.14E-05	9.66E-13	1.13E-02	NS	4.31E-07	2.96E-10	4.94E-17	NS
MGLL	4.90E-11	6.02E-46	2.05E-02	8.72E-24	4.79E-02	1.37E-03	1.33E-22	NS	2.57E-21	3.27E-02	2.38E-12	1.53E-15	2.31E-31	2.99E-02	3.74E-05	1.55E-05	9.14E-07	1.45E-02	5.21E-07
MSRA	7.13E-05	1.91E-49	2.15E-06	4.22E-14	5.80E-05	2.11E-02	2.15E-17	8.65E-09	7.74E-08	4.80E-09	1.68E-22	4.08E-19	1.34E-30	1.71E-02	6.22E-06	3.87E-04	2.55E-10	7.84E-12	3.88E-02
PEMT	5.19E-05	8.23E-20	2.15E-06	2.19E-15	1.92E-02	9.00E-04	2.92E-02	9.87E-04	2.86E-02	1.47E-06	3.51E-22	4.40E-02	NS	NS	4.59E-03	4.77E-05	NS	3.71E-15	2.11E-03
RGN	1.61E-09	4.13E-52	2.15E-06	2.35E-06	7.82E-06	NS	2.07E-14	7.61E-03	4.79E-19	8.57E-10	3.80E-19	4.48E-17	1.02E-30	1.61E-03	4.03E-15	1.57E-03	3.31E-10	4.50E-11	1.32E-16
UGP2	2.17E-05	1.36E-46	2.15E-06	2.41E-26	1.14E-04	1.58E-02	6.27E-16	2.62E-03	4.67E-15	6.75E-05	3.98E-20	4.26E-05	NS	1.50E-03	1.49E-09	1.31E-07	4.34E-08	5.93E-08	1.22E-05

Figure A.3: The 15 genes that are the basis for the breast cancer subnetwork were analyzed across 24 different cancer types. The 19 cancer types where the majority of the 15 genes are down-regulated are depicted here. Significant values in blue denote statistically significant lower expression in pooled cancer tissue when compared to pooled normal tissue, values in red denote statistically higher expression in cancer, and NS values denote a non-significant change in gene expression between cancer and normal tissues. All P-values were calculated using the Mann-Whitney U test.

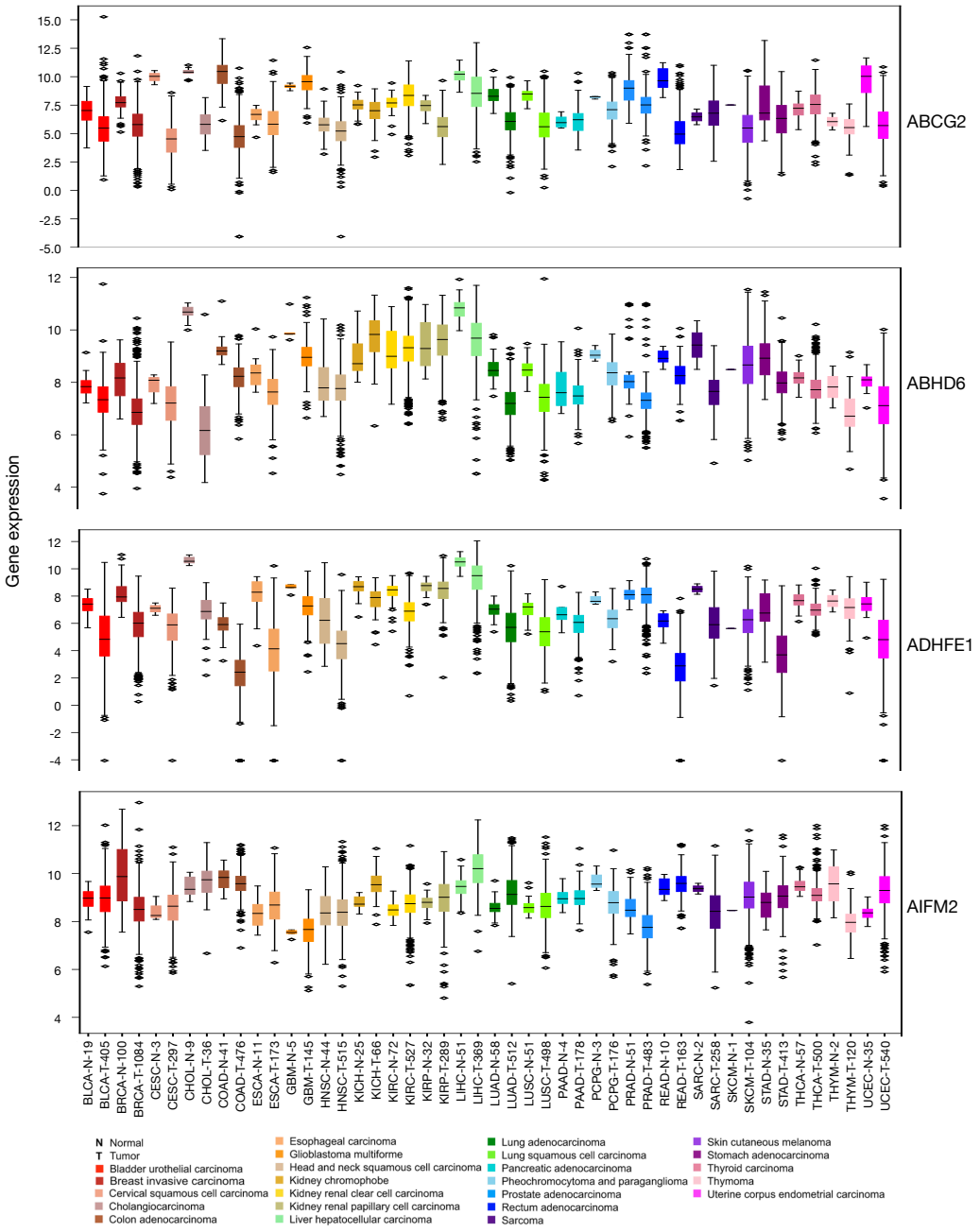


Figure A.4: Gene expression calculated by RNA-seq by Expectation-Maximization (log2) for *ABCG2*, *ABHD6*, *ADHFE1*, and *AIFM2* for 24 different cancer types comparing cancer and normal samples within the same tissue type. Box plots are colour-coded in pairs based on cancer type where the boxplot on the left is gene expression in cancer and the plot on the right is gene expression in normal tissue. Labels on the x-axis represent the abbreviation of the cancer type, whether the sample represents tumor (T) or normal (N) tissue, and the sample size.

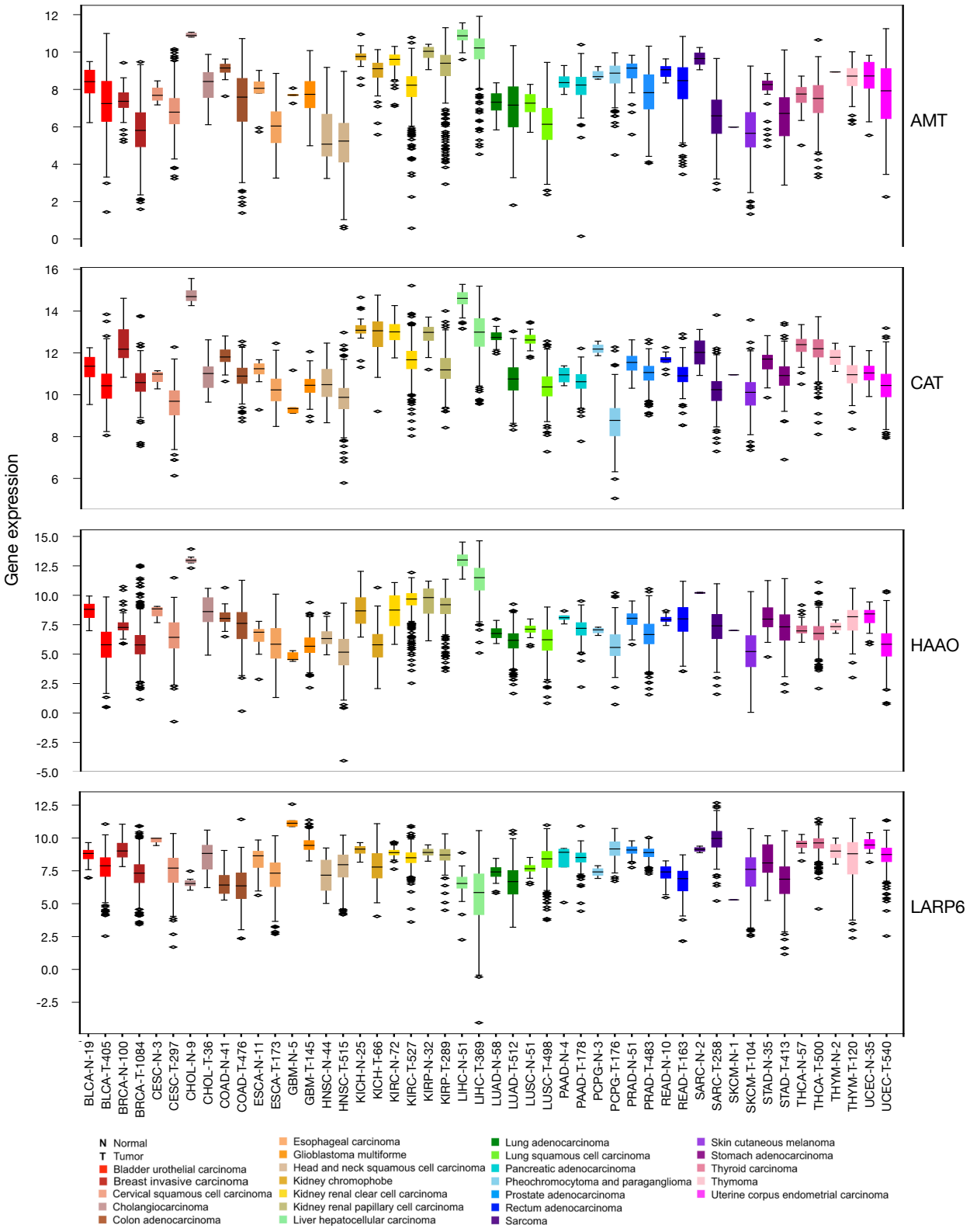


Figure A.5: Same as description for Figure A.4, except for *AMT*, *CAT*, *HAAO*, and *LARP6*.

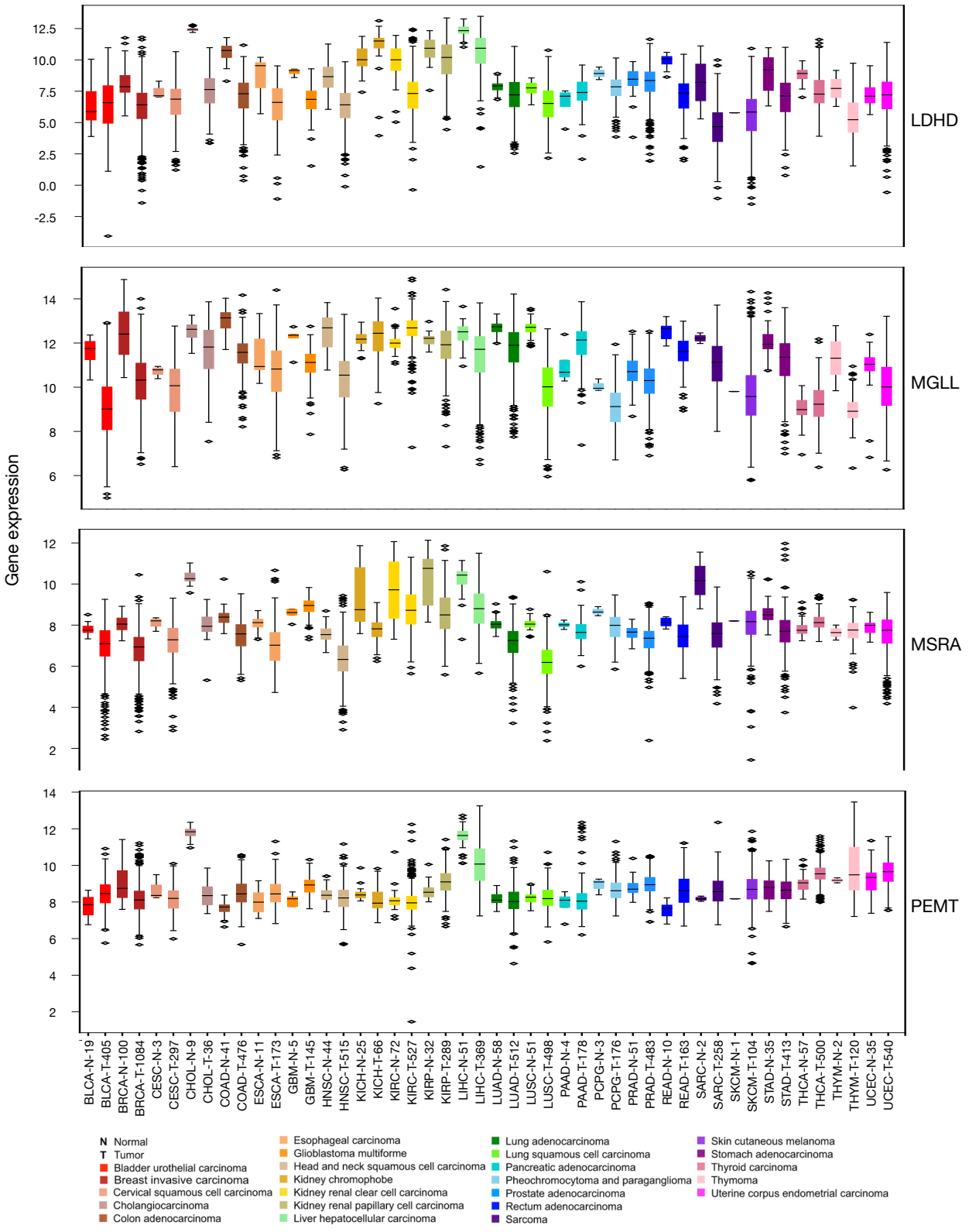


Figure A.6: Same as description for Figure A.4, except for *LDHD*, *MGLL*, *MSRA*, and *PEMT*.

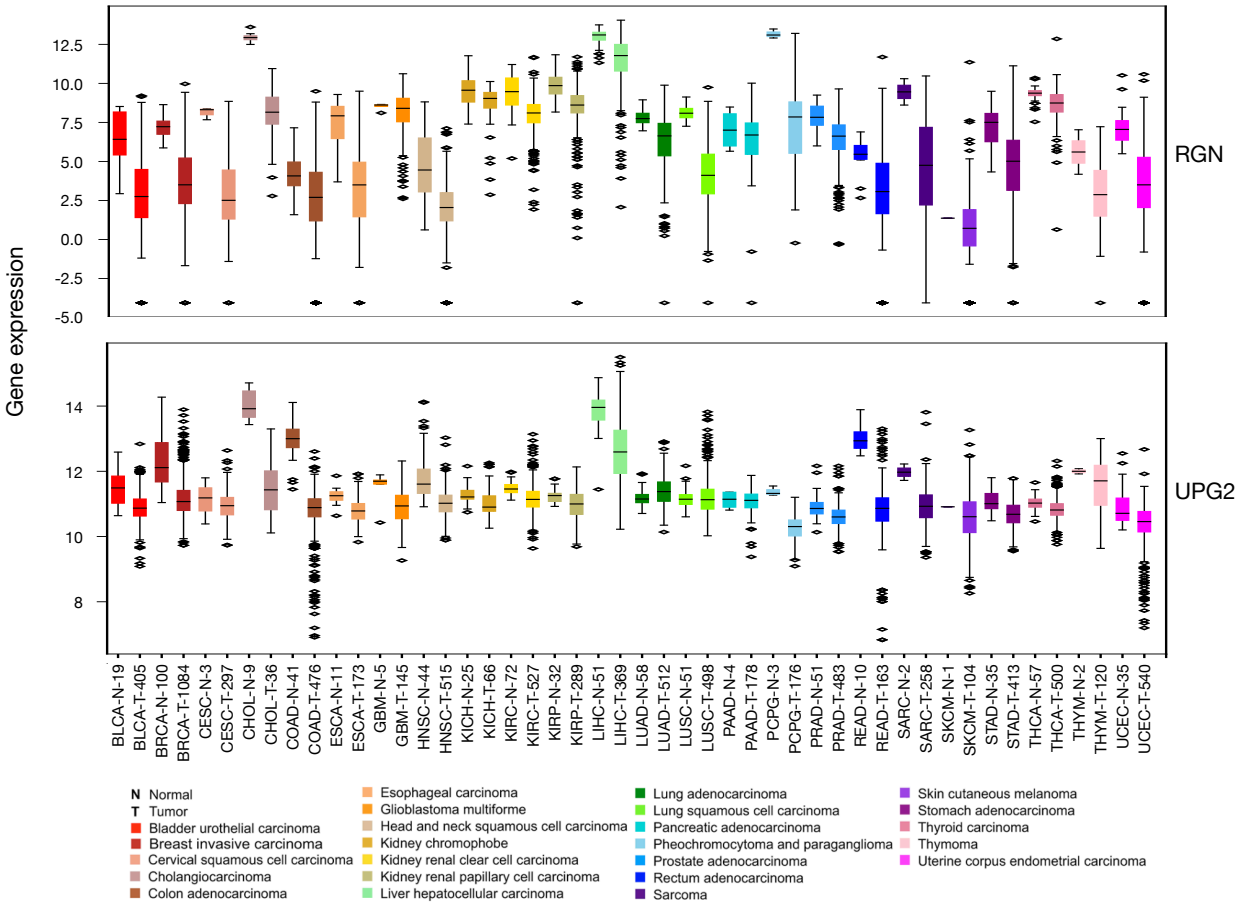


Figure A.7: Same as description for Figure A.4, except for *RGN* and *UPG2*.

Appendix B

Supplementary Tables

Supplementary tables included as comma separated value (csv) files. Table legends for each are provided below.

Supplementary Table B.1: Strict one-to-one ortholog mapping for yeast genes in humans. Ortholog mapping information was taken from InParanoid and filtered to identify instances where one yeast protein mapped to one human protein. The resulting 1,266 pairs of orthologs can be found in this table.

Supplementary Table B.2: HYGIN network table. This table contains the initial mapping data from the yeast network where SL interactions are represented in pairs by A and B (Yeast Gene ID A, Yeast Gene ID B, Yeast Gene Name A, Yeast Gene Name B, eValue, and pValue). Through the 3-stage mapping process used to generate HYGIN, it also contains columns corresponding to Yeast Protein Accession (A and B), Human Protein Accession (A and B), and Human Gene Name (A and B). Human gene names were used to generate the network in Cytoscape. Due to some reciprocal screens in the initial yeast screen, there were some duplicate (reciprocal) entries in this table. Once the network was loaded into Cytoscape,"Remove duplicate edges" was used to eliminate reciprocal hits. This resulted in the 1,009 genes and 10,419 edges in the final HYGIN network.

Supplementary Table B.3: GO Slim mapping table. Twenty-two GO Slims were used for colouring the HYGIN network in Cytoscape. These terms were curated by and courtesy of the Vizeacoumar research group. The common name for the human protein and its accession number accompany the GO Slim terms that have been curated by the Vizeacoumar research group. *DDR Pathways = "DNA Damage and Repair Pathway" and NA Metabolism = "Nucleic Acid Metabolism".

Supplementary Table B.4: List of genes that were identified as down-regulated in breast cancer at a 2-fold cut-off and with a Wilcoxon Signed Rank P-value less than 0.05. Mean expression is the average of the log(2) ratio (cancer gene expression/normal gene expression) for each gene. P-values are from Wilcoxon Signed Rank test (Python) after adjustment (Benjamini-Hochberg procedure), and log(10)(P-values) were used to generate the volcano plot in Figure 5.3.

Supplementary Table B.5: Genetic interactions in the breast cancer subnetwork. The table contains a list of the SL gene pairs that make up the breast cancer specific subnetwork where column GeneA represents the genes that are down-regulated in breast cancer, and column GeneB are their SL gene pairs.

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C.3 Figure 2.3

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